

ESTIMATION OF THE RELATIVE BIOAVAILABILITY OF ZINC
FROM REAGENT AND FEED GRADE ZINC SOURCES
FOR SHEEP AND POULTRY

By

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To my parents, Manuel J. and Maria L., because they gave
me their best inheritance, education.

To my wife Evi L., and my daughters, Evelyn C. and Mary S.,
for their love and permanent support.

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Abstract of Dissertation Presented to the Graduate School
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Nine experiments (EXP) were conducted to investigate the relative bioavailability of Zn (BVZN) for sheep and poultry fed practical diets. Tissue Zn concentration (TZN) was used as the response variable of BVZN. In EXP 1 and 2, sheep were fed high levels of Zn (HZN) for 23 d; EXP 1 was conducted to estimate the BVZN from reagent grade (RG) sulfate, carbonate, oxide and metal; BVZN index based on TZN from liver, kidney and pancreas was 100%, 106%, 105% and 76%, respectively. In EXP 2, BVZN from feed grade (FG) sulfate-A, sulfate-B, oxide-A and oxide-B were estimated to be 100%, 86%, 87% and 79%, respectively. Liver metallothionein (MT) increased as dietary Zn intake (DZNI) increased. In EXP 3 and 4, chicks were fed HZN from RG and FG sources for 20 and 21 d, respectively. Bone Zn (BZN)

adjusted for DZNI estimated values of 100%, 57%, 54% and 30% for RG sulfate, carbonate, oxide and metal, respectively, in EXP 3. In EXP 4, BVZN from RG sulfate, FG sulfate A and B, and FG oxide A and B was 100%, 78%, 74%, 52% and 38%, respectively. In EXP 5, chicks fed low levels of Zn from RG sulfate, FG sulfate-A and oxide-A for 20 d had BVZN values of 100%, 93%, and 68%, respectively. In EXP 6, chicks at 14 d of age were crop intubated (CRTUB) with a high oral dose of Zn from RG sulfate, acetate and chloride; FI decreased and TZN increased at 24 h post oral dose. In EXP 7, chicks were given HZN either in the diet or CRTUB from 2 to 3 wk of age. The BVZN from RG sulfate, acetate and chloride for chicks given HZN in the diet was 100%, 106% and 101%, respectively. For CRTUB chicks, the BVZN from RG sulfate, acetate, chloride and FG gluconate was 100%, 118%, 105% and 105%, respectively. In EXP 8, chicks fed HZN from RG sulfate or FG gluconate for 21 d had BVZN of 100% and 109%. In EXP 9, chicks were fed HZN from RG acetate and RG oxide for 3 wk; BZN increased as DZNI increased. Chicks fed RG acetate had higher liver MT and pancreas MT than those fed RG oxide. These studies indicate TZN can be used to determine BVZN from RG and FG sources for sheep and poultry.

CHAPTER 1 INTRODUCTION

Zinc (Zn) is a bluish-white metal that is derived from numerous mineral ores, the principal one being the sulfide sphalerite, sometimes called zinc blend (NRC, 1979). Zinc was shown to be an essential nutrient for lower forms of life more than a century ago (Raulin, 1869) and for plants in 1926 (Sommer and Lipmann, 1926).

Todd et al. (1934) reported the first evidence that Zn was necessary for growth and health in rats and mice. Despite the initial evidence that Zn was required by rats it was thought that Zn was abundant in foodstuffs and a deficiency of Zn was unlikely to occur in domestic animals under normal conditions.

Tucker and Salmon (1955) presented the first clear evidence that Zn prevented and cured parakeratosis in pigs. This disease, which caused great economic losses during the late 1940s and early 1950s occurred with swine fed diets inadequate in dietary Zn and with excess of Ca. This discovery of the pig's need for Zn encouraged animal scientists to study the nutritional physiology of Zn in food-producing animals.

O'Dell and Savage (1957) showed that Zn was required for growth, feathering and skeletal development in the chick. Shortly after, Prasad et al. (1961) reported the first evidence of Zn deficiency in humans in the Middle East.

Keilin and Mann (1940) reported the first Zn-metalloenzyme, carbonic anhydrase; since then a variety of Zn-responsive pathological conditions have been observed in animals that were thought to be related to other Zn-metalloenzymes. It is the involvement of Zn via metalloenzymes which makes this element play its most important role in biological systems. Lack of dietary Zn has been shown to impair DNA synthesis, protein metabolism and growth in animals, particularly when the cells of a particular system are dividing, or growing (Riordan and Vallee, 1976; Chesters and Arthur, 1988).

In many countries around the world there are large areas of Zn-deficient soils and pastures in which cattle showed typical signs of Zn deficiency (Legg and Sears, 1960), and significant responses have been demonstrated to mineral supplementation containing Zn (Masters and Fels, 1985).

O'Dell and Savage (1960) presented evidence that phytic acid, which is found in plant seeds, binds Zn and decreases its availability. The recognition of this relationship between phytic acid and Zn is essential in nonruminant

nutrition, especially in poultry nutrition because the practical diets commonly used now consist mainly of corn and soybean meal. With these constituents the Zn content of practical diets may be limiting unless supplemented. During the last 20 years most research has been conducted to estimate bioavailability of Zn from foodstuffs used in human consumption not supplemental Zn sources.

Bioavailability of Zn may be measured using quantifiable response variables, such as growth (weight), skeletal development (mineralization), and tissue Zn concentration among other physiological functions (Fox et al., 1981; Wedekind et al., 1991). Early work conducted by Edwards (1959) suggested that no differences were observed in Zn bioavailability among sulfates, oxides and carbonates of Zn; weight gain was used as the response variable to measure Zn bioavailability in these studies.

Miller et al. (1981), working with young growing pigs, reported that availability of Zn from metallic dust was 30% to 33% greater than the availability of Zn from Zn oxide, which was set as the standard source. Wedekind and Baker (1990), working with chicks, reported bioavailability values for feed grade Zn oxide of 44.1% compared to Zn sulfate reagent grade; total bone Zn was used as response variable. Spears (1989) working with heifers reported no differences in Zn bioavailability between Zn methionine and Zn oxide using weight gain, feed intake, feed efficiency, plasma Zn

or plasma alkaline phosphatase as indices of Zn bioavailability.

It has been indicated that several feed-grade Zn sources are finding their way into the animal industry, but Zn bioavailability of these sources is not completely known. Therefore, it is necessary to have this information for more precise feed formulation.

Ott et al. (1965) reported that Zn accumulated in tissues of lambs fed high dietary levels of Zn; these findings suggested that this technique of measuring tissue accumulation may be useful to measure Zn bioavailability. Subsequently, Black et al. (1984a, b), working with manganese (Mn), demonstrated that tissue uptake of Mn from high-level, short-term supplementation of dietary Mn was a useful measure of bioavailability of inorganic sources.

The experiments reported herein were conducted to determine if tissue Zn concentration in sheep and chicks fed high dietary levels of Zn could be used as primary response variable for determining relative biological availability of Zn from reagent grade and feed-grade sources.

CHAPTER 2 REVIEW OF LITERATURE

Significance of Zinc as an Essential Nutrient

Zinc has been shown to be an essential nutrient for more than a hundred years. Raulin (1869) demonstrated the essentiality of Zn for the fungus Aspergillus niger, but it was not until the first two decades of this century that Zn was shown to be required for higher plants (Sommer and Lipmann, 1926).

The first clear evidence that Zn is required by animals was reported by Todd et al. (1934), who produced Zn deficiency in rats. Following the original report, other researchers corroborated the essentiality of Zn for the rat (Stirn et al., 1935; Hove et al., 1937). Despite the initial evidence that Zn was required by rats fed purified diets, it was thought that Zn was abundant in foodstuffs and a deficiency of Zn was unlikely to occur in domestic animals and humans.

Tucker and Salmon (1955) demonstrated the importance of Zn supplementation for domestic animals, when it was shown that parakeratosis in swine was caused by inadequate dietary Zn. Subsequently, O'Dell and Savage (1957) showed that Zn

is required for growth, feathering, and skeletal development in poultry.

Zinc deficiency in humans of the Middle East was first documented in the early 1960s by Prasad et al. (1961). Although great strides have been made in elucidating the essentiality of Zn, a great frontier of research remains.

Chemistry

Zinc is a bluish-white metal with atomic number 30 and an atomic weight of 65.38. Characteristic properties are specific gravity of 7.13 g/cm at 20°C, melting point of 419.5°C and boiling point of 906°C. Zinc is derived from numerous minerals, but the principal mineral ore is the sulfide sphalerite, sometimes called Zn blend. Sphalerite is the source of most metallic zinc (NRC, 1979).

Zinc exists in a hydrated form in neutral and acidic aqueous solutions, but the hydroxide is precipitated in alkaline solution. Zinc compounds possess chemical properties that make them useful as oxides, sulfates, carbonates, sulfides, borates, phosphates, chlorides, cyanides, nitrates, permanganates, peroxides and organic complexes (NRC, 1979).

Zinc has five naturally occurring stable isotopes-- ^{64}Zn , ^{66}Zn , ^{67}Zn , ^{68}Zn , and ^{70}Zn -- and three radioisotopes-- ^{65}Zn ($t_{1/2} = 245$ d), ^{69}Zn ($t_{1/2} = 13.8$ h), and ^{63}Zn ($t_{1/2} = 38.1$ min)-- which have been applied as tracers in biological

research (Cotton and Wilkinson, 1988). Zinc is in group IIB of the periodic table and is a divalent element (Zn^{2+}) with the electron configuration of 2-8-18-2. The Zn^{2+} ion does not share many of the common characteristics of most transition elements (Douglas et al., 1983). For example, Zn^{2+} is diamagnetic rather than paramagnetic because all of its electrons are paired. Also Zn^{2+} will not form colored compounds since all of its subshells are filled and light cannot shift an electron from one energy level to another.

One characteristic that Zn shares with transition elements is the ability to bind ligands noncovalently to form coordination complexes. This property is biologically important because ligands dictate the absorbability of Zn, facilitate the transport and storage of Zn and impart to Zn the ability to perform essential biological functions (Nebergall et al., 1976).

There are a lot of ubiquitous ligands that bind Zn tightly and play physiological roles in the body; these include aspartate, glutamate, lysine, histidine, tyrosine, cysteine, arginine and N-terminal amino acids (Hughes, 1984). These amino acids contain N, S, and O, which are good Lewis bases (Basolo and Pearson, 1967). When several different ligands are present, competitive equilibria result and a variety of Zn complexes are formed. The Zn^{2+} can be chelated and precipitated by a number of chelating agents,

including some natural constituents of the diet (Vohra and Kratzer, 1966).

Method of Analysis

Vallee and Galdes (1984) indicated that Zn is included among the trace elements because early investigators were not able to quantify Zn in biological samples because of the very low levels present. Advances in methodology, instrumentation and analytical chemistry have made possible more accurate study of the occurrence of Zn in biological systems. The most widely used method of measuring Zn in biological samples for nutritional or biochemical studies is air-acetylene flame atomic absorption spectrophotometry.

The Perkin-Elmer (1982) recommended guidelines for standard atomic absorption conditions for Zn are a wavelength of 213.9 nm, which provides a sensitivity of .018 mg/L with excellent accuracy and precision. Preparation of samples for analysis involves combustion of organic material (wet or dry ashing), followed by solubilization of the ash in acid (Fick et al., 1979).

Standardization of the atomic absorption procedure is usually against National Institute of Standards and Technology biological reference materials (bovine liver, citrus leaves). Standards of similar viscosity and with matrix composition that include other major minerals

comparable with the test samples are used to calibrate the instrument.

The increased necessity of determining many elements at all concentration levels, and particularly at lower concentrations, has led to the successful evolution of inductively coupled plasma-atomic emission spectrophotometry (ICP-AES). The method can be used as an alternative to atomic absorption to analyze Zn and other metals (Varma, 1991).

Biochemistry of Zinc

The biochemical basis for the essentiality of Zn is not completely understood. Although Zn is known to be an essential cofactor for a variety of Zn metalloenzymes (Vallee and Galdes, 1984), Zn has also been postulated to have other roles in gene expression (Vallee and Falchuk, 1981; Wu and Wu, 1987), in membrane structure and function (Bettger and O'Dell, 1981), as a second messenger and as a protective/trigger agent in molecular storage systems (Grummt et al., 1986), and for improvement of the stability of human growth hormone (Cunningham et al., 1991), functions that may be independent of Zn metalloenzyme activity. The reader is referred to other references for more detailed information (Vallee and Galdes, 1984; Hambidge et al., 1986; Mills, 1987; Chesters, 1989; Williams, 1989; Bray and

Bettger, 1990). However, some of the vital roles of Zn in animal nutrition will be presented herein.

Zinc Metalloenzymes

The most important biological function of Zn is as a constituent of metalloenzymes. Zinc metalloenzymes can be categorized as serving either catalytic, structural, regulatory or non-catalytic roles (Huber and Gershoff, 1973; Vallee and Galles, 1984). The first enzyme confirmed as a Zn metalloenzyme was carbonic anhydrase (Keilin and Mann, 1940). Since then, the list of known Zn metalloenzymes contains members of all six enzyme classes (Valle and Galles, 1984). These classes and representative enzymes include oxidoreductases (alcohol dehydrogenase, superoxide dismutase), transferases (RNA nucleotidyl transferases), hydrolases (alkaline phosphatase, angiotest-in-converting enzyme, carboxypeptidase), lysases (carbonic anhydrase, δ -aminolevulinic acid dehydratase), isomerases (phosphomannose isomerase) and ligases (t-RNA synthetase, pyruvate carboxylase). In these enzymes Zn provides structural integrity and/or participates in catalysis. It is the involvement of Zn in a wide variety of metabolic pathways via Zn metalloenzymes that probably account for some of the physiological signs associated with nutritional Zn deficiency.

Cell Division and Tissue Growth

Williams and Mills (1970) reported that young rats offered a Zn-deficient diet showed a marked reduction in growth after only a few days on the diet and before a major reduction in total body Zn content or concentration had occurred. Similar results in reduction of food intake and growth rate were observed in chicks (O'Dell et al. 1958; Morrison and Sarett, 1958). In experiments conducted with rats, Chesters and Quarterman (1970) reported also an abrupt reduction in food intake to a level which, in pair-fed animals, severely restricts growth. However, when the Zn intake of deficient rats was increased by gavage to that of controls offered Zn-adequate diet ad libitum, the animals failed to grow and became ill (Masters et al., 1983). Therefore, the reduced tissue growth of a Zn-deficient animal results from a Zn-responsive biochemical defect rather than from a physiological effect of loss of appetite.

Grummt et al. (1986) indicated that the dinucleotide 5',5'-diadenosine tetraphosphate (DAT) acts as a secondary messenger, serving as an intermediate signal molecule between membrane events resulting from mitogenic stimulation and the subsequent initiation of nuclear DNA synthesis. Synthesis of DAT in both bacterial (Goerlich and Holler, 1984) and eukaryotic systems (Brevet et al., 1982) was markedly enhanced by the addition of Zn ions (Zn^{2+}). Lack of dietary Zn has been shown to inhibit growth and, in

particular, to impair DNA synthesis in animals; this seems to result from a reduced ability to initiate the events leading to DNA synthesis rather than from an impairment of its synthesis per se (Chesters and Arthur, 1988).

Tissue growth can be partitioned between increase in cell size and in cell number, only the latter being associated with net synthesis of DNA. Williams and Chesters (1970) and Dreosti et al. (1972) showed that in liver, kidneys, spleen and testes, DNA synthesis was more severely affected by lack of Zn than was protein synthesis during the onset of deficiency in rats. These observations suggest that DNA synthesis and cell division are more susceptible to lack of Zn than are increase in protein mass and cell size.

Several key enzymes required for nucleic acid synthesis and degradation are Zn-dependent. These Zn-dependent enzymes include the potentially rate-limiting enzymes involved in DNA synthesis, that is, DNA polymerase (Vallee and Galles, 1984), aspartate transcarbamylase (Hambidge et al., 1986), and thymidine kinase (Dreosti and Hurley, 1975).

Zinc Deficiency

Todd et al. (1934) were the first to demonstrate that Zn was an essential component in the diet of mammals. More complete and detailed information about biochemical pathologies of Zn deficiency in domestic animals and humans is found in other reports (Miller, 1970; NRC, 1979;

Sandstead, 1981; Hambidge et al., 1986; NRC, 1985; Solomons, 1986; Clegg et al., 1989; Suttle and Jones, 1989).

Clinical Manifestations

It has been shown that animals which consume Zn-deficient diets eventually develop a variety of pathologies, including anorexia, retardation or cessation of growth, abnormal immune function, hypogeusia, impaired reproduction capacity, lesions of the integument and its outgrowths--hair, wool, feathers (Underwood, 1981; Scott et al., 1982; NRC, 1984; NRC, 1985; Scott, 1986; Apgar and Everett, 1991; Bentley and Grubb, 1991).

Impaired growth is the clinical feature that is universally observed in Zn deficiency, and this may reflect the association with early depression of appetite (Underwood, 1981; Scott et al., 1982). Early studies showed depressed growth rates when Zn was limited in the diet of the chick (O'Dell et al., 1958; Young et al., 1958; Morrison and Sarett, 1958; Roberson and Schaible, 1958). Similar results have been observed in turkeys (Kratzer et al., 1958; Supplee et al., 1958), Japanese quail (Fox and Harrison, 1964), pheasant chicks (Scott et al., 1959) and ducklings (Wight and Dewar, 1975).

O'Dell and Savage (1957) reported that abnormal leg bone development was observed in chicks which received no supplemental Zn. Shortening and thickening of the tibia and

tarso-metatarsus as well as enlargement of the hock were observed in Zn-deficient chicks. This disorder may be related to impaired development of epiphyseal cartilage (Westmoreland and Hoekstra, 1969; Nielsen et al., 1970; Suwarnasarn, 1982) or defective collagen synthesis (McClain et al., 1973).

Poor feather development is also a clear characteristic of Zn deficiency in chicks fed purified diets (O'Dell et al., 1958; Berg et al., 1963). Experiments conducted with pullets fed practical diets corroborated findings obtained with chicks (Sunde, 1972). The investigator reported that addition of 52 mg Zn per kg diet to the basal diet decreased the incidence of feather abnormality.

Tucker and Salmon (1955) demonstrated that parakeratosis was a Zn deficiency disorder in swine. Also Zn-deficient chicks developed severe skin lesions on the feet and legs and around the eyes (O'Dell et al., 1958; Roberson and Schaible, 1958). Parakeratosis in grazing cattle was first reported by Legg and Sears (1960) and is the most obvious manifestation of Zn deficiency in cattle (Ott et al., 1964; Miller, 1970). It is claimed that these epidermal abnormalities may be related to decreased amino acid incorporation into skin proteins (Hsu and Anthony, 1971), reduced glycolytic enzyme activity (Im et al., 1975) or defective collagen crosslinking (McClain et al., 1973).

The effects, including lesions, of a Zn deficiency in sheep are similar to those observed in cattle (Miller et al., 1964; Mills et al., 1967). In sheep the wool becomes loose and brittle, loses its crimp and often is lost. The horns and hooves of sheep may be soft, deformed and lack normal striations (Miller, 1970).

Zinc Requirements

It is recognized that Zn must be present in the diet of all animals, including humans. In the practical feeding of domestic animals, it is recommended that Zn be supplied in the diet more or less continuously, because animals have only small amounts of readily available Zn stored in the body (Hambidge et al., 1986). Normally, Zn present in body tissues cannot be utilized by the animal to meet its need for zinc.

The exact requirement of domestic animals is very important for the estimation of bioavailability because homeostatic mechanisms limit absorption at higher levels of Zn intake (Bremner and Beattie, 1990). It is also known that the animal may be able to make some adjustments to a low Zn intake through decreased excretion of Zn and perhaps by increased absorption efficiencies (Evans et al., 1973; Miller, 1973).

Zinc requirement for domestic animals is affected by many physiological and dietary factors including age, stage

or level of production, and the presence or absence of dietary inhibitors or enhancers of Zn absorption (Solomons and Cousins, 1984).

In general, the Zn requirement of animals fed purified diets is lower than that of those fed practical diets (O'Dell et al., 1972). The experiments described in this report use the growing chick as a model for nonruminants, and the lamb as a model for ruminants, hence the requirement levels of chickens and sheep are described below.

Chicken

The Zn requirement of young chickens fed purified diets containing egg albumen or casein was reported to be between 12 and 20 mg of Zn (as ZnCl_2) per kg diet (O'Dell and Savage, 1960; Zeigler et al., 1961). The requirement was reported to be higher if the diet contained soybean protein (Morrison and Sarett, 1958).

Turk et al. (1959) reported that laying hens fed purified diets were reported to require about 15 mg Zn per kg diet. On the other hand, when laying hens were fed practical diets the Zn requirement was over 50 mg/kg as Zn carbonate for optimal feathering and progeny (Berg et al., 1963) and for maximal pullet growth (Sunde, 1972).

The NRC (1984) recommendations for broilers and laying and breeding hens fed practical diets are 40, 50 and 65 mg Zn per kg diet, respectively.

Sheep

Ott et al. (1965) reported that a diet containing 18 mg Zn as ZnO per kg diet (dry basis) did not support maximal live weight gain of lambs fed a purified diet. Mills et al. (1967) estimated that 7.7 mg Zn per kg diet was sufficient to meet growth requirements of lambs but did not maintain the plasma Zn concentration within the normal range.

Underwood and Somers (1969) reported that a diet supplying 17.4 mg Zn per kg diet (dry basis) was adequate for growth and for maintenance of normal appetite, but this level was not adequate to permit normal testicular development and spermatogenesis. Further work conducted by Pond (1983) reported that a Zn concentration of 19 to 26 mg Zn per kg diet (dry basis) was adequate for growth of lambs.

The NRC (1985) suggests that minimal requirements are 20 mg Zn per kg diet (dry basis) for growth and 33 mg Zn per kg diet (dry basis) for maintenance of normal reproductive function in males and for pregnancy and lactation in females.

Zinc Metabolism

Absorption

A wide variety of approaches has been used to study the site of Zn absorption in the digestive tract of domestic animals such as in vivo techniques (Naveh et al., 1988;

Hempe and Cousins, 1989) and in vitro techniques (Davies, 1980; Smith and Cousins, 1980; Oestreicher and Cousins, 1989). Despite considerable research neither the site nor the mechanism of Zn absorption has been completely delineated (Cousins, 1985b; Lonnerdal, 1989; Hempe and Cousins, 1991).

Most reports indicate that the small intestine is the primary site of Zn absorption and that little Zn is absorbed from the stomach or from the large intestine (Miller and Crager, 1965). The mechanism and control of Zn absorption are complex processes; that more sensitive methods in molecular biology will eventually lead to the elucidation of these mechanisms (Cousins, 1985a; King, 1990; Hempe and Cousins, 1991).

Zinc absorption is influenced by a number of chemical (Seal and Heaton, 1983) and environmental factors (Solomons and Cousins, 1984). Absorption of Zn is 5 to 10% in rats (Feaster et al., 1955), 20 to 30% in growing chicks (Suso and Edwards, 1971), and 50.8% in humans (Spencer et al., 1966). The rate of Zn absorption is highly variable in ruminants ranging from less than 10% to more than 80% of that consumed (Miller, 1970).

The relative contribution of the duodenum, jejunum, and ileum toward overall Zn absorption is not clear. It has been reported that Zn absorption is 60% from the duodenum, 30% from the ileum and 10% from the jejunum in fasted rats

(Davies and Olpin, 1979), but Antonson et al. (1979) reported that Zn absorption is 60% from the ileum and 20% from the jejunum and duodenum in nonfasted rats.

Several investigators (Davies, 1980; Steel and Cousins, 1985; Hoadley et al., 1987) have reported that absorption involves two kinetic processes, a passive diffusion and carrier-mediated components that may represent paracellular and intracellular absorption pathways. Carrier-mediated Zn absorption is increased during periods of low Zn intake, suggesting up-regulation of the carrier system (Hoadley et al., 1987). In contrast, the diffusion component of Zn absorption is unaffected by Zn deficiency, and absorption via this process is proportional to luminal Zn concentration. It is not known how much Zn can be absorbed via this process (Cousins, 1985b).

Cousins (1985a) has proposed four phases for Zn absorption: a) uptake by the intestinal cells, b) transfer through mucosal cells, c) entry to the portal system and d) re-secretion of Zn by intestinal cells. Several reports indicate a potential function of various intracellular Zn-binding ligands in controlling Zn absorption, however, this has not been completely elucidated. Recently Hempe and Cousins (1991, 1992) reported that they have identified a low molecular cysteine-rich intestinal protein (CRIP) in rat intestinal mucosa that could function as an intracellular Zn carrier. The same investigators indicated that the protein

was not detected in liver or pancreas, suggesting a role specific to the intestine.

Homeostatic Regulation

The body content of zinc can be controlled by re-excretion and by homeostatic regulation of intestinal uptake (Evans et al., 1973). Absorption decreases as the intraluminal concentration of the Zn ion (Zn^{2+}) increases (Steel and Cousins, 1985). A key role in this process was suggested for metallothionein (MT). Homeostatic regulation of zinc absorption appears to be related to synthesis of intestinal MT (Menard et al., 1981; Vallee and Galdes, 1984; Cousins, 1985b; Hempe et al., 1991).

Metallothionein

Metallothioneins (MTs) are low-molecular weight cytosolic proteins found ubiquitously in eukaryotic species. A unique characteristic of MTs is that they bind stoichiometric quantities of heavy metals such as Cd, Cu and Zn. This unique property of MT has been investigated to identify the role of MT in Zn and Cu metabolism (Hamer, 1986; Dunn et al., 1987; Richards, 1989a, b; Bremner and Beattie, 1990; McCormick, 1991).

Metallothionein has been isolated and characterized from a variety of livestock species including calves (Bremner and Marshall, 1974), sheep (Whanger et al., 1981a,

b), horses (Kagi et al., 1974), pigs (Bremner and Young, 1976), and chickens (McCormick et al., 1988).

A variety of homeostatic roles has been proposed for MT; for instance, it may function as a mucosal block to Zn absorption (Cousins, 1979). It has been hypothesized that release of Zn from the intestine to portal circulation increases when cellular MT concentrations are low as occurs in Zn deficiency (Hoadley et al., 1988). Oestreicher and Cousins (1985) and Cousins and Lee-Ambrose (1992) indicated that during high Zn intake, Zn absorption decreases and intestinal MT increases. Furthermore intestinal MT has been reported to act in the detoxification of heavy metals and in the homeostatic control of Zn and Cu (Bremner and Beattie, 1990).

Dunn et al. (1987) reported that metal binding reduces MT degradation and increases stability of the protein. Thus intracellular concentration of MT is dependent upon Zn. When dietary Zn was very low, intracellular levels of Zn were reduced and MT in some tissues was degraded (Cousins, 1985b).

Physicochemical Characteristics

Metallothioneins are single-chain polypeptides of 60 to 61 amino acids with a low molecular weight of 6000 to 7000 Da and have a high content of heavy metals, 7 to 12 metal atoms/molecule protein (Dunn et al., 1987; Kagi and

Schaffer, 1988). The primary structure of MTs is characterized by a high content of cysteine ranging from 23 to 33% with no disulfide bonds, histidine, or aromatic amino acids (Dunn et al., 1987; Richards and Steele 1987).

Metallothioneins contain two globular domains (Furey et al., 1986): an α domain within the carboxyl terminal end of the molecule extending from amino acid 31 to 61 and an amino terminal β domain extending from residue 1 to 30. The β domain contains 9 cysteine residues and binds either 3 Zn^{2+} or Cd^{2+} ions or 6 Cu^{1+} ions (Nielson et al., 1985). The α domain contains 11 cysteine residues and binds either 4 Zn^{2+} or Cd^{2+} ions or 5 Cu^{1+} . The relative affinities of MTs for the metals are $\text{Cu}^{1+} > \text{Cd}^{2+} > \text{Zn}^{2+}$, however Zn^{2+} is the most physiologically ubiquitous (Hamer, 1986). Each Zn^{2+} is bound to MT by 4 cysteine thiolate ligands in a tetrahedral coordination complex (Hamer, 1986).

Transport

The Zn released by the intestinal cells at the basolateral-serosal surface into the mesenteric capillary is carried in the portal blood to the liver (Cousins, 1989). Albumin appears to be the principal metabolically active exchangeable Zn pool in blood, with approximately 80% of the Zn plasma. Several other Zn-containing components of plasma have been reported, among them α_2 -macroglobulin, (Parisi and

Vallee, 1970), transferrin (Charlwood, 1979), and amino acids, especially cysteine and histidine (Morgan, 1981).

Excretion

Under normal conditions feces constitute the major route for Zn excretion (Weigand and Kirchgessner, 1980), however, 2 to 10% may be recovered in urine. Fecal excretion includes both unabsorbed dietary Zn and endogenous fecal Zn excretion; the latter is primary a mechanism of Zn homeostasis. It has been reported that endogenous fecal Zn excretion increased with increased dietary Zn (Miller, 1973) and decreased on low Zn diets (Hambidge et al., 1986). Secretion of Zn into the gastrointestinal tract occurs via pancreatic and biliary secretions, desquamation of intestinal cells, and possibly serosal to mucosal transfer either via intracellular or paracellular pathways (Cousins, 1985a).

Urinary losses appear to change relatively little with the needs of the animal (Miller, 1975). They may be influenced more by other metabolic activities, such as tissue breakdown during starvation or disease of the animal, than by the animal's zinc needs. Since urine and feces are eliminated together in avian species, there is little information relative to zinc excretion in the aves.

Nutritional Interactions

The effects of excess Zn intake and its interaction with other nutrients for humans and domestic animals have been reviewed (NRC, 1979, 1980, 1985; Fox and Jacobs, 1986; Hambidge et al., 1986). Thus a detailed examination of this extensive area is beyond the scope of this review.

The literature indicates that the severity of adverse effects is related to Zn exposure level and duration; animal age, sex, species and nutritional status; and composition of the diet (Underwood, 1981; Williams et al., 1989). Hill and Matrone (1970) postulated that the configuration and coordination chemistry influence the mechanism of transport of elements with similar physicochemical properties, therefore they may compete for common pathways. Coordination chemistry plays an important role in the behavior of the essential trace elements, especially Zn and Cu. The Zn ion has 10 outer electrons in the d orbital and forms a tetrahedral sp^3 chelate configuration and prefers a coordination number of four (Cotton and Wilkinson, 1988). These characteristics are identical to those of Cu^{1+} and Cd^{2+} , thus, these elements would be expected to interact with Zn.

Competitive interactions between chemically similar elements may cause detrimental effects on their metabolism, especially if one of the elements is essential and present at marginal levels in the diet (Blalock and Hill, 1988).

Dewar et al. (1983) observed lesions of the pancreas and gizzard in laying hens fed high Zn diets (1000 mg/kg) for 4 wk. The investigators suggested that was due to Zn-Cu interaction.

Calcium

Animal studies have shown that high dietary Ca reduces the intestinal absorption of Zn (Heth and Hoeskstra, 1965), and decreases Zn bioavailability (O'Dell et al., 1972). The detrimental effects of high Ca are most evident in diets containing phytic acid (O'Dell, 1972). Heth et al. (1966) indicated that high dietary levels of Ca reduced Zn absorption in the presence of high dietary phosphate, and suggested that adsorption on Ca phosphate is responsible for the reduced Zn availability for rats. Vohra and Kratzer (1966) also showed that Ca phosphate reduced Zn availability for turkey poults. However, Zn bioavailability for sheep (Sword et al., 1984) was reported not to be affected by dietary Ca phosphate.

Copper

Large quantities of ingested Zn can interfere with Cu absorption and, as Cu is necessary for Fe metabolism, anemia may result (O'Neil-Cutting et al., 1981). There is a strong negative interaction between Zn and Cu (Rosa et al., 1986; Dewar et al., 1983) but the effect of excess Zn or Cu

metabolism is much more significant than the opposite; Cu is in practice no serious inhibitor of Zn absorption. Several investigators have presented evidence that Cu deficiency results in anemia by mechanisms other than decreased ceruloplasmin activity (Weisenburg et al., 1980; Stahl et al., 1989).

The type of diet besides the level of Cu plays an important role in the Zn-Cu interaction. When animals were fed purified diets high in Zn and low in Cu, a decrease was observed in liver and kidney Cu concentration (Stahl et al., 1989; Blalock and Hill, 1988). On the other hand, chicks fed practical diets supplemented with high levels of Zn did not show similar responses as those chicks fed purified diets (Johnson et al., 1962; Jackson et al., 1986).

Iron

Excess Zn intake has been shown to decrease tissue Fe levels as well as induce anemia in chicks (Bafundo et al., 1984), quail (Hamilton et al., 1981), swine (Cox and Hale, 1962) and rats (Magee and Matrone, 1960). It has been claimed that excess Zn ingestion interferes with Fe utilization possibly by directly inhibiting Fe absorption (Solomons and Jacobs, 1981; Siewicki et al., 1986). Because more Fe than Cu is present in practical diets, competition between Zn and Fe has greater potential impact on Zn

metabolism. Tissue levels may be more sensitive to changes in Zn intake than anemia (O'Neil-Cutting et al., 1981).

Zinc Distribution in Tissues

Hambidge et al. (1986) reviewed tissue Zn concentration in most mammalian tissues and showed that tissue Zn levels were fairly constant among species. Turk (1965) reported levels of Zn in tissues of chicks and the values were fairly similar to those observed in mammals. The Zn concentrations of most soft tissues were relatively constant at most intake levels and were not greatly affected by Zn deficiency (Turk, 1965). Tissues that are most sensitive to excess dietary Zn intake are bone, liver, kidney, pancreas, and small intestine (Ott et al., 1966c, d; Kincaid et al., 1976a, b).

The concentrations of trace elements in tissues are sometimes used as indicators of Zn bioavailability in chicks, sheep and rats (Henry et al., 1988; Moncilovic, 1975; Wedekind et al., 1991).

Bioavailability

Chemical determination of the total content of an element in a diet does not indicate the amount of the element that is available to meet the physiological requirements of domestic animals. This is because frequently only a portion of a chemically determinable element in food can be absorbed and converted in the various

tissues to its metabolically active form. As a result of this the concept of bioavailability has come into use.

Fox et al. (1981) defined bioavailability as a quantitative measure of the utilization of a nutrient under specified conditions necessary to support the organism's normal structure and physiological processes. O'Dell (1985) defined bioavailability as the proportion of a nutrient in a feedstuff that can be absorbed and utilized. A definition proposed by Forbes (1984) indicates that bioavailability reflects the efficiency with which consumed nutrients are absorbed from the digestive tract and thus are available for storage or use.

O'Dell et al. (1972) reported that Zn present in foods of plant origin, particularly those derived from oilseeds is almost unavailable for chicks. A detailed description of bioavailability of Zn in natural products (plants and animals) is beyond the scope of this report.

Factors Affecting Bioavailability

The biological availability of trace elements depends on both intrinsic and extrinsic factors (O'Dell, 1983). Intrinsic factors include species and genotype (Kincaid, 1976a, b), age (Schisler and Kienholz, 1967), stage of production (Berg et al., 1963), nutritional status (Stuart et al., 1986), a physiological stress (Turk and Stephens, 1967; Orr et al., 1990), and intestinal microflora and

infection (Bafundo et al., 1984). The intrinsic factors affect bioavailability but are of physiological nature that cannot be controlled many times. The extrinsic or dietary factors that affect bioavailability of Zn may include Zn-deficient diets, presence of phytate in the diets, solubility of the source, mineral interactions, protein content of the diet, and chelators present in the diet (Smith and Embling, 1984; Stuart et al., 1986; Shafey et al., 1991; Vohra and Kratzer, 1964).

O'Dell and Savage (1960) presented evidence that phytic acid, which is found in plant seeds, binds Zn and decreases its availability. When phytate was added to either casein or gelatin or soybean diets limiting in Zn, the growth of chicks was severely depressed.

In general the effect of the extrinsic factors is more important than the intrinsic factors because they can be manipulated or controlled to improve bioavailability of Zn. During the last 20 years extensive research has been conducted to estimate bioavailability of nutrients in foodstuffs used in human consumption. However, studies in animals have shown that bioavailability differs markedly with different concentrated forms of an element (Ammerman and Miller, 1972) with elements supplied by different foods (O'Dell et al., 1972), different inorganic sources (Spears, 1989; Wedekind and Baker, 1990), and organic sources (Hill et al., 1986; Wedekind et al., 1991; Pimentel et al., 1991).

Assessment of Bioavailability

The use of animal models to study bioavailability of Zn has two objectives 1) to identify the factors that influence bioavailability of Zn and 2) to estimate the amount of bioavailable Zn in a source for domestic animals. O'Dell (1983) indicated that the best way to assess bioavailability is to compare absorption and utilization of the element in a feedstuff with those in a standard soluble salt of the element. Fox et al. (1981) indicated that the relative biological availability value is bioavailability expressed as a percentage of the value obtained when the nutrient is fed in the form of a reference material.

Bioavailability is measured using response variables; primary indices of response include quantifiable levels of morphology or physiological function that indicate health status (Forbes, 1984). Examples are growth (weight, height, etc), skeletal development (bone size, conformation and mineralization), hematopoiesis, and ability to perform work (Fox et al., 1981; O'Dell, 1983; Edwards, 1959). Secondary indices of responses are quantifiable responses that are not direct measures of health status but must be correlated with primary indices under defined conditions. Examples include, whole body retention of the element or the level of inorganic elements, metabolites, enzymes or hormones in tissues, body fluids or excretory products (Fox et al., 1981).

Some methods used to assess bioavailability of trace elements only measure absorption or uptake by cells and provide estimates of bioavailability (Black et al., 1984a, b; Moncilovic et al., 1975; Amine et al., 1972; Wedekind et al., 1991).

Studies of Zn bioavailability have been conducted using in vivo and in vitro techniques (Lease and Williams, 1967; Hill et al., 1987). Description of all methods is beyond the scope of this report. However, the most used methods are 1) plasma zinc response (Kincaid 1979), 2) perfusion techniques (Steel and Cousins, 1985), 3) Intestinal strips and loops (Reinhold et al., 1974), 4) brush border membrane vesicles (Menard and Cousins, 1983), 5) in vitro ligand-binding assays (Jones et al., 1985), 6) tissue Zn concentration (Zeigler et al., 1964), 7) metabolic balance studies (Solomons, 1982), 8) radioisotope methods (Evans et al., 1979), 9) stable isotopes (Turnlund et al., 1982), 10) intrinsic versus extrinsic labeling techniques (Fairweather-Tait et al., 1991), 11) dual isotope methods (Flanagan et al., 1985), 12) standard curve estimation (O'Dell, 1972), and 13) slope ratio assay (Moncilovic et al., 1975; Wedekind et al., 1991).

With regard to slope ratio assay, this method is used to estimate Zn bioavailability as the ratio of the slopes of the two response curves (Finney, 1978). The methodology and statistics of slope ratio assays were reviewed by Finney

(1978). This method is a true bioassay and has been used widely in Canada (Moncilovic et al., 1975; Shah and Belonje, 1984), at the University of Illinois (Forbes and Parker, 1977; Wedekind et al., 1991) and at the University of Florida (Henry et al., 1987a, b; Black et al., 1984a, b).

The indicators of bioavailability that are used with slope ratio assays include circulating Zn and tissue Zn concentration or total tissue Zn (Wedekind et al., 1991; Kincaid, 1979). One important aspect besides the response variable is that the use of slope ratio assays dictates that the standard and test response curves must have a common y-intercept for fundamental assay validity (Ware et al., 1983).

CHAPTER 3
BIOLOGICAL AVAILABILITY OF ZINC FROM INORGANIC REAGENT AND
FEED GRADE ZINC SOURCES FED AT HIGH DIETARY LEVELS TO
SHEEP, EXPERIMENTS 1 AND 2

Introduction

Zinc (Zn) has been demonstrated to be an essential nutrient for a variety of animal species (Underwood, 1981; Hambidge et al., 1986). While severe deficiency of dietary Zn is uncommon in animals except under controlled experimental conditions, there is now convincing evidence that a marginal Zn deficiency is by no means rare (Legg and Sears, 1960). Effects of a mild Zn deficiency in ruminants include decreased feed intake, growth, feed efficiency, milk production, resistance to infection and stress and lower reproductive efficiency (Miller, 1970).

Dietary supplementation of Zn is especially important for ruminants because it has improved growth rate, reproductive functions such as spermatogenesis and testosterone biosynthesis (Hatch et al., 1987; Apgar, 1985; Underwood and Somers, 1969; Underwood, 1981). Ammerman and Miller (1972) indicated that an evaluation of the bioavailability of different inorganic sources of Zn has only been performed with poultry. Recently the work of Wedekind and Baker (1990) showed bioavailability values for

feed grade ZnO of 44.1% compared to ZnSO_4 , set at 100% determined by multiple regression slope-ratio methodology, using tibia Zn accumulation in chicks. Spears (1989) working with heifers reported no differences in Zn bioavailability between Zn oxide and Zn methionine using weight gain, feed intake, feed efficiency, plasma Zn or plasma alkaline phosphatase as indices of Zn bioavailability. The two predominant sources of Zn used by the animal industry are Zn Oxide (72% Zn) and Zn sulfate (36% Zn); therefore it is necessary to evaluate different sources that are currently being used by the feed industry.

Black et al. (1984a, b) working with manganese (Mn) demonstrated that tissue uptake of Mn from high level, short term supplementation of dietary Mn was a useful measure of bioavailability of inorganic sources. Reports of Zn accumulation in the tissues of lambs fed high levels of Zn (Ott et al., 1966) suggest that this technique may be useful to measure Zn bioavailability. The purpose of the present research was to determine the bioavailability (BV) of Zn from inorganic reagent grade (RG) and feed grade (FG) sources of Zn for sheep, fed high dietary Zn concentrations, using tissue Zn uptake as the primary response variable.

Materials and Methods

Experiment 1

This study compared the BV of Zn from reagent grade (RG) sources, Zn sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), Zn basic carbonate ($3\text{Zn}(\text{OH})_2 \cdot 2\text{ZnCO}_3$), Zn oxide (ZnO) and Zn metal when added to a practical corn-soybean meal-cotton seed hulls diet adequate in Zn. Forty-two crossbred wether lambs averaging 40 kg were assigned randomly to one of seven treatments in a completely randomized design. Prior to the experiment, lambs were group-fed a commercial corn-soybean meal-cotton seed hulls diet at approximately 800 g/hd daily and grass hay was available ad libitum.

The basal diet was a corn-soybean meal-cotton seed hulls diet (44 mg/kg Zn, DM basis by analysis, Table 1-1). The diets were formulated to meet the requirements for growing lambs (NRC, 1985) and were supplemented with 0, 700, 1400 or 2100 mg/kg Zn from RG sulfate or 1400 mg/kg Zn from carbonate, oxide and metal, added at the expense of cornstarch. Lambs were housed in individual wooden pens with expanded metal floors in an open side barn. Feed intake was restricted to 1000 g/hd daily (as-fed basis) and tap water was available ad libitum. Lambs were fed the treatment diets for 21 d following a 7-d adjustment period during which all animals were fed the basal diet. At the

end of the experiment, lambs were stunned with a captive bolt shot and killed by exsanguination.

Liver, both kidneys and pancreas were excised and frozen for further mineral analyses. Calcium, Mg, Zn, Cu, Fe and Mn in diets and sources, and Zn, Cu and Fe in tissues collected were determined by flame atomic absorption spectrophotometry on a Perkin-Elmer Model 5000 with AS-50 autosampler (Perkin-Elmer Corporation, 1982). Standards were matched for macroelements and acid concentrations as needed and standard reference material from the National Institute of Standards and Technology was included with samples. Phosphorus (P) in diets and sources was measured by a colorimetric method (Harris and Papat, 1954). Relative solubility following 1 h of constant stirring at 37°C of .1 g of each source in 100 ml of either neutral ammonium citrate, 2% citric acid, .4% HCl, or water (Watson et al., 1970), magnetic susceptibility (Watson et al., 1971), and chemical and physical characteristics of Zn sources were determined, and X-ray diffraction patterns were interpreted.

Tissue Zn concentrations were analyzed by one-way ANOVA and multiple linear regression (MLR) analysis were performed using the general linear model (GLM) procedure (SAS, 1985). A log 10 transformation of all tissue Zn concentrations was made to account for heterogeneity of variances. Zinc BV was determined using $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ as a standard by means of MLR and slope-ratio methodology (Finney, 1978). Standard errors

TABLE 1-1. COMPOSITION OF BASAL DIET, EXP. 1 AND 2

Item	%	
	Exp. 1	Exp. 2
Ingredient composition ^a		
Corn, ground yellow	58.95	58.95
Cotton seed hulls	21.00	21.00
Soybean meal, solv-ext	12.00	12.00
Alfalfa meal	3.00	3.00
Corn oil	2.00	2.00
Corn starch ^b	1.50	1.50
Salt, trace mineralized ^c	1.00	1.00
Limestone, ground	.55	.55
Microingredients ^d	+	+
TOTAL	100.00	100.00
Chemical composition ^e		
Dry matter, %	88.60	89.20
Crude protein, %	12.00	12.00
Ca, %	.42	.41
P, %	.31	.33
Mg, %	.14	.14
Zn, mg/kg	44	64
Cu, mg/kg	6	10
Fe, mg/kg	106	123
Mn, mg/kg	39	50

^aAs-fed basis.^bZn supplements added at expense of corn starch.^cTo supply the following Zn, mg/kg (ZnCO₃); Cu, 10 mg/kg (CuCl₂); Fe, 110 mg/kg (FeSO₄·7H₂O); Mn, 36 mg/kg (MnO); Co, 2.5 mg/kg (CoCO₃); I, 2.5 mg/kg (KI).^dVitamin A, 2200 USP units/kg; vitamin D₃, 440 IU/kg; vitamin E, 15 IU/kg; etoxyquin, 187 mg/kg; Se, .2 mg/kg (Na₂SO₃).^eDry matter basis; crude protein calculated, dry matter and minerals determined by analysis.

were calculated for each regression coefficient.

Experiment 2

The objectives of this study were to: 1) compare the BV of Zn in feed grade (FG) sources including zinc ZnSO_4 -granular (sulfate-A), ZnSO_4 -spray dried (sulfate-B), and two ZnO (oxide-A and oxide-B) when added at high levels to a practical corn-soybean meal-cotton seed hulls diet and 2) determine the effect of these Zn sources on metallothionein (MT) accumulation in liver of sheep fed high levels of Zn.

Forty-two crossbred wether lambs averaging 37.5 kg were allotted randomly to seven treatment groups in a completely randomized design. The basal diet was a corn-soybean meal-cotton seed hulls diet (64 mg/kg Zn, DM basis by analysis, Table 1-1). The diets were formulated to meet requirements for growing lambs (NRC, 1985) and were supplemented with 0, 700, 1400 or 2100 mg/kg Zn from FG ZnSO_4 -A, or 1400 mg/kg Zn from ZnSO_4 -B, ZnO-A and ZnO-B, added at the expense of cornstarch. Husbandry of lambs, tissue collection and statistical analyses were similar to Experiment 1.

Metallothionein was measured in liver by the $^{109}\text{Cd}^{2+}$ -hemoglobin affinity assay (Eaton and Toal, 1982). About 1 g of hepatic tissue was homogenized with 3 volumes of cold 10mM Tris-HCl buffer (pH 7.4), with a Potter-Elvehjem glass-Teflon tissue grinder, and centrifuged (40,000 x g; 30 min; 4°C). Then the supernatant was heated (100°C; 5 min) and

centrifuged (10,000 x g; 5 min). Next, 200 μ l of cadmium solution (2 μ g Cd and .5 μ Ci ^{109}Cd per ml of 10 mM Tris-HCl buffer, pH 7.4) was added to a 200 μ l aliquot of the supernatant and the mixture was incubated (10 min; room temperature). Finally, 100 μ l of 2% hemoglobin was added. Then the sample was heated (100°C; 2 min) and centrifuged (10,000 x g; 5 min). After this step was repeated, the concentration of MT was calculated using ^{109}Cd measurements with a Gamma Spectrometer Model 4000 (Beckman Instruments, Inc.) of supernatants from the sample and appropriate controls.

Results

Experiment 1

Chemical and physical characteristics of mineral sources were determined and are presented in Table 1-2. Analysis of the RG sources indicated Zn concentrations of 22.0, 58.5, 77.4 and 95.5% for the sulfate, carbonate, oxide and metal sources, respectively. The sulfate and metal sources had greater amounts of small particles than the carbonate and metal sources, respectively.

The sulfate was completely soluble in all four solutions, while the carbonate and oxide sources were completely soluble in neutral ammonium citrate, 2% citric acid and .4% hydrochloric acid but insoluble in water. The metal source was the least soluble of the sources tested,

TABLE 1-2. CHEMICAL AND PHYSICAL CHARACTERISTICS OF REAGENT GRADE ZINC SOURCES, EXP. 1

	Zinc source (RG)			
	Sulfate	Carbonate	Oxide	Metal
Chemical constituents, Dry matter basis				
Zn, %	22.0	58.5	77.4	95.5
Ca, %	.28	.31	.27	.27
P, mg/kg	ND ^a	ND	ND	ND
Mg, mg/kg	1.2	ND	ND	11.3
Cu, mg/kg	ND	ND	46.2	75.5
Fe, mg/kg	ND	ND	ND	ND
Mn, mg/kg	ND	ND	ND	37.8
Particle size, % ^b				
+ 20	--	15.8	14.2	--
+ 100	38.1	80.9	85.8	58.2
+ 200	46.6	3.3	--	16.2
- 200	15.3	--	--	24.6
Physical appearance	White crystals	White powder	White powder	Dark gray powder
Magnetic susceptibility, %	--	--	--	4.2

TABLE 1-2-- Continued

Relative solubility, % ^c	Zinc source (RG)			
	Sulfate	Carbonate	Oxide	Metal
Neutral ammonium citrate	100	100	93.7	5.9
Citric acid, 2%	100	100	96.1	10.9
HCl, .4%	100	100	95.0	29.5
Water	100	4.2	1.0	.1
Interpretation of X-ray patterns ^d	$\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ (38%); $\text{Zn}_2\text{CO}_3(\text{OH})_6 \cdot \text{H}_2\text{O}$ ZnO (100%) $\text{ZnSO}_4 \cdot 6\text{H}_2\text{O}$ (58%); (55%); Zn metal $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (4%); ZnO (40%); (99%); possibly a $\text{Zn}_5(\text{CO}_3)_2(\text{OH})_6$ ZnO (1%) trace of (5%) $(\text{Fe}, \text{Zn})\text{SO}_4 \cdot 6\text{H}_2\text{O}$			

^aNot detected.^bRetained by a No. 20 (850 μm), 100 (150 μm) or 200 (75 μm) or passing through a 200 (75 μm), respectively.^cSolubility in solvents at 37°C for 1 h with constant stirring expressed as % of total mineral content.^dAnalyzed by Florida Institute of Phosphate Research, Bartow, Florida.

being insoluble in water and with solubilities of 5.9, 10.9 and 29.5% in neutral ammonium citrate, 2% citric acid and .4% hydrochloric acid, respectively. The relatively low magnetic susceptibility observed in all sources tested may be related to the lack of Fe content of the sources.

Interpretation of X-ray diffraction patterns indicated a high degree of purity for the oxide and metal sources, while the sulfate and carbonate differed from their theoretical form (Table 1-2). Analysis of lamb performance (Table 1-3) indicated that high dietary Zn concentration had no effect on final body weight. All animals consumed the entire daily feed allotment of 1.0 kg.

Liver mineral concentrations are presented in Table 1-4. Supplementation of Zn as Zn sulfate at 2100 mg/kg resulted in no further significant increase in Zn uptake in any of the tissues analyzed; therefore this level was not included in any of the MLR analysis. Liver Zn was increased ($P<.0001$) by dietary Zn intake. Liver Cu and Fe were not affected by dietary Zn intake. Multiple linear regression of log transformed liver Zn (Table 1-5) on dietary Zn concentration indicated that the increase was linear ($P<.0001$, $R^2=.77$). Zinc and Cu concentrations in kidney were affected ($P<.0001$) by dietary Zn intake (Table 1-6). Kidney Fe was not affected by Zn source. Kidney Cu increased with increasing dietary Zn.

TABLE 1-3. PERFORMANCE OF LAMBS FED A PRACTICAL DIET SUPPLEMENTED WITH REAGENT GRADE ZINC SOURCES, EXP. 1

Zinc source ^a	Added Zn	Daily Intake	Body weight	
			Initial	Final
	mg/kg	kg	kg	kg
Basal	0	1.0	41.6	41.0
Sulfate	700	1.0	41.0	41.5
	1400	1.0	41.0	41.0
	2100	1.0	41.0	40.3
Carbonate	1400	1.0	39.5	40.8
Oxide	1400	1.0	40.4	40.5
Metal	1400	1.0	39.6	40.5
SE ^b		-	.5	.4
ANOVA,	P value			
Source			.76	.98

Note: Each value represents six lambs. Animals were fed the experimental diets for 23 d.

^aBasal diet contained 44 mg/kg Zn dry basis by analysis.

^bPooled standard error.

TABLE 1-4. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION OF LIVER IN LAMBS, EXP. 1

Zinc source ^a	Added Zn, mg/kg	Liver, mg/kg ^b		
		Zn	Cu	Fe
Basal	0	97	227	146
Sulfate	700	355	189	140
	1400	468	231	123
	2100	524	199	135
Carbonate	1400	608	165	117
Oxide	1400	580	225	141
Metal	1400	342	262	116
SE ^c		20.9	12.7	5.7
ANOVA,	P value			
Source		.0001	.507	.66

Note: Each value represents six lambs. Animals fed 23 d.

^aBasal diet contained 44 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cPooled standard error.

TABLE 1-5. MULTIPLE REGRESSION OF TISSUE ZINC CONCENTRATION ON DIETARY ZINC CONCENTRATION, EXP. 1

Zinc source ^b	Tissue ^a		
	Liver	Kidney	Pancreas
	Reg. Equation ^c slope $\pm$ SE (10^{-5})		
Sulfate	48.19 $\pm$ 6.2	75.96 $\pm$ 7.3	81.14 $\pm$ 7.1
Carbonate	49.96 $\pm$ 5.9	86.48 $\pm$ 7.0	82.44 $\pm$ 6.8
Oxide	49.42 $\pm$ 5.9	88.97 $\pm$ 7.0	79.56 $\pm$ 6.8
Metal	32.70 $\pm$ 5.9	60.49 $\pm$ 7.0	65.47 $\pm$ 6.8
b_0	2.06	2.04	1.99
R^2	.77	.92	.87
SD	.15	.14	.17
P	.0001	.0001	.0001

Note: Each value represents six lambs. Animals fed 23 d.

^aDry matter basis.

^bBasal diet contained, 44 mg/kg Zn dry basis by analysis.

^cRegression equation based on log 10 transformation of tissue Zn concentration, level 2100 mg/kg Zn from the Zn sulfate source was not included in the MLR analysis.

TABLE 1-6. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION OF KIDNEY IN LAMBS, EXP. 1

Zinc source ^b	Added Zn, mg/kg	Kidney, mg/kg ^a		
		Zn	Cu	Fe
Basal	0	101	22.6	151
Sulfate	700	498	38.2	141
	1400	1177	45.9	168
	2100	1985	62.2	190
Carbonate	1400	1890	66.6	158
Oxide	1400	2036	58.9	184
Metal	1400	816	37.5	162
SE ^c		60.4	1.4	7.7
ANOVA,	P value			
Source		.0001	.0001	.60

Note: Each value represents six lambs. Animals fed 23 d.

^aDry matter basis.

^bBasal diet contained 44 mg/kg Zn dry basis by analysis.

^cPooled standard error.

Multiple linear regression of log transformed kidney Zn on dietary Zn by source indicated that the increase was linear ($P<.0001$; $R^2=.92$).

The effect of dietary Zn concentration and source on pancreas mineral concentration is summarized in Table 1-7. Pancreas Zn and Cu were increased ($P<.0001$) by dietary Zn source. However, pancreas Fe was not affected by dietary treatments. Multiple linear regression of log transformed pancreas Zn on dietary Zn by source indicated that the increase was linear ($P<.0001$; $R^2=.87$).

Evaluation of tissues with respect to Zn uptake indicated that uptake was related linearly to dietary Zn. The linear increase in liver, kidney and pancreas Zn as dietary Zn increased allows its use as a bioassay with Zn sulfate the standard source set at 100%. The relative biological availability (BV) values, using liver Zn concentration, (Table 1-8) were 103.7, 102.5 and 67.9% for the carbonate, oxide and metal sources, respectively.

When kidney Zn concentration was used as the response variable, the BV values were 113.8, 117.1 and 79.6% for the carbonate, oxide and metal sources, respectively (Table 1-9). The BV values based on pancreas Zn concentration (Table 1-10) were 101.6, 98 and 80.7% for the carbonate, oxide and metal sources, respectively.

TABLE 1-7. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION OF PANCREAS IN LAMBS, EXP. 1

Zinc source ^b	Added Zn, mg/kg	Pancreas, mg/kg ^a		
		Zn	Cu	Fe
Basal	0	88	8.8	79.3
Sulfate	700	502	10.8	79.3
	1400	1267	17.0	62.0
	2100	1224	21.5	67.0
Carbonate	1400	1488	21.3	61.5
Oxide	1400	1232	26.9	71.4
Metal	1400	910	12.1	86.6
SE ^c		60.5	.9	2.9
ANOVA,	P value			
Source		.0001	.0001	.178

Note: Each value represents six lambs. Animals fed 23 d.

^aDry matter basis.

^bBasal diet contained 44 mg/kg Zn dry basis by analysis.

^cPooled standard error.

TABLE 1-8. RELATIVE BIOLOGICAL AVAILABILITY OF REAGENT GRADE ZINC SOURCES BASED ON MULTIPLE REGRESSION OF LIVER ZINC ON DIETARY ZINC CONCENTRATION, EXP. 1

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^d %
Sulfate	48.19 $\pm$ 6.2 ^a	100.0 $\pm$ --	-
Carbonate	49.96 $\pm$ 5.9 ^a	103.7 $\pm$ 12.5	79 - 128
Oxide	49.42 $\pm$ 5.9 ^a	102.5 $\pm$ 12.4	78 - 127
Metal	32.70 $\pm$ 5.9 ^a	67.9 $\pm$ 10.7	47 - 89

^aBasal diet contained 44 mg/kg Zn dry basis by analysis.

^bRegression equation based on log 10 transformation of liver Zn, values have been multiplied by 10⁻⁵, slopes in the same column with the same superscript are not significantly different (P<.05).

^cEstimated relative biological availability (BV) value of zinc sources determined by slope ratio methodology.

^dConfidence interval at 95% for BV of zinc sources.

TABLE 1-9. RELATIVE BIOLOGICAL AVAILABILITY OF REAGENT GRADE ZINC SOURCES BASED ON MULTIPLE REGRESSION OF KIDNEY ZINC ON DIETARY ZINC CONCENTRATION, EXP. 1

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^d %
Sulfate	75.96 $\pm$ 7.3 ^a	100.0 $\pm$ --	-
Carbonate	86.48 $\pm$ 7.0 ^a	113.8 $\pm$ 9.7	95 - 133
Oxide	88.97 $\pm$ 7.0 ^a	117.1 $\pm$ 9.9	92 - 131
Metal	60.49 $\pm$ 7.0 ^a	79.6 $\pm$ 8.2	63 - 96

^aBasal diet contained 44 mg/kg Zn dry basis by analysis.

^bRegression equation based on log 10 transformation of kidney Zn, values have been multiplied by 10⁻⁵, slopes in the same column with the same superscript are not significantly different (P<.05).

^cEstimated relative biological availability (BV) value of zinc sources determined by slope ratio methodology.

^dConfidence interval at 95% for BV of zinc sources.

TABLE 1-10. RELATIVE BIOLOGICAL AVAILABILITY OF REAGENT GRADE ZINC SOURCES BASED ON MULTIPLE REGRESSION OF PANCREAS ZINC ON DIETARY ZINC CONCENTRATION, EXP. 1

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^d %
Sulfate	81.14 $\pm$ 7.1 ^a	100.0 $\pm$ --	-
Carbonate	82.44 $\pm$ 6.8 ^a	101.6 $\pm$ 10.8	80 - 123
Oxide	79.56 $\pm$ 6.8 ^a	98.0 $\pm$ 10.4	78 - 118
Metal	65.47 $\pm$ 6.8 ^a	80.7 $\pm$ 9.3	62 - 99

^aBasal diet contained 44 mg/kg Zn dry basis by analysis.

^bRegression equation based on log 10 transformation of pancreas Zn, values have been multiplied by 10^{-5} , slopes in the same column with the same superscript are not significantly different ($P < .05$).

^cEstimated relative biological availability (BV) value of zinc sources determined by slope ratio methodology.

^dConfidence interval at 95% for BV of zinc sources.

A relative biological availability index, calculated using the mean of the estimates from the different tissues, provided values of 106.4, 105.8 and 76.0% for the carbonate, oxide and metal sources, respectively.

Experiment 2

Chemical and physical characteristics of zinc sources were determined and are presented in Table 2-1. Analysis of the FG sources indicated Zn concentrations of 33.0, 32.2, 72.0 and 71.0% for the sulfate-A, sulfate-B, oxide-A and oxide-B, respectively.

The sulfate-A had greater amounts of large particles than the sulfate-B, and the two Zn oxide sources had almost similar particle sizes. The two sulfate sources were completely soluble in neutral ammonium citrate, 2% citric acid, .4% hydrochloric acid and water. The oxide-A and oxide-B were quite soluble in .4% hydrochloric acid but less soluble in neutral ammonium citrate and 2% citric acid, and almost completely insoluble in water.

The feed grade Zn sources tested showed very low magnetic susceptibility which may be related to the lack of Fe content of the sources. Interpretation of X-ray diffraction patterns indicated a high degree of purity in Zn content for the FG sources under study.

Feed intake and final body weight were not affected by dietary treatment (Table 2-2). All animals under study

TABLE 2-1. CHEMICAL AND PHYSICAL CHARACTERISTICS OF FEED GRADE ZINC SOURCES, EXP. 2

	Zinc source (FG)			
	Sulfate-A	Sulfate-B	Oxide-A	Oxide-B
Chemical constituents				
Dry matter basis				
Zn, %	33.0	32.2	72.0	71.0
Ca, %	.44	.40	1.3	2.2
P, %	ND ^a	ND	ND	ND
Mg, mg/kg	18.2	46.9	69.1	68.1
Cu, mg/kg	36.5	ND	409	1205
Fe, mg/kg	1386	537	32650	24710
Mn, mg/kg	177	1612	865	381
Particle size, % ^b				
+ 20	24.9	--	1.1	1.2
+ 100	74.9	39.1	98.9	95.6
+ 200	.2	21.4	--	3.2
- 200	--	39.5	--	--
Physical appearance				
	White granular	White powder	Brownish green powder	Dark gray powder
Magnetic susceptibility, %				
	--	--	1.7	7.0

TABLE 2-1-- Continued

	Zinc source (FG)			
	Sulfate-A	Sulfate-B	Oxide-A	Oxide-B
Relative solubility, % ^c				
Neutral ammonium citrate	100	100	53.0	81.9
Citric acid, 2%	100	100	69.1	85.7
HCl, .4%	100	100	98.6	97.8
Water	100	100	.6	.2
Interpretation of X-ray patterns ^d	ZnSO ₄ ·H ₂ O (100%)	ZnSO ₄ ·H ₂ O (76%) ZnSO ₄ (24%)	ZnO (100%)	ZnO (93%) CaCO ₃ (7%)

^aNot detected.

^bRetained by a No. 20 (850 μ m), 100 (150 μ m) or 200 (75 μ m) or passing through a 200 (75 μ m), respectively.

^cSolubility in solvents at 37°C for 1 h with constant stirring expressed as % of total mineral content.

^dAnalyzed by Florida Institute of Phosphate Research, Bartow, Florida.

consumed the entire daily feed allotment of 1.0 kg.

Liver mineral concentration is presented in Table 2-3. Supplementation of Zn as $\text{ZnSO}_4\text{-A}$ at 2100 mg/kg resulted in no further significant increase in Zn concentration of any of the tissues analyzed; therefore this level was not included in any of the regression analysis.

Dietary Zn increased ($P<.0001$) hepatic Zn, liver Cu was also increased ($P<.056$) but to lesser degree than liver Zn. However, liver Fe uptake was decreased ($P<.012$) by intake of high levels of Zn. Metallothionein concentration in liver increased ($P<.0001$) as dietary Zn increased (Table 2-3). Liver Zn and liver MT were highly correlated ($r=.96$).

Multiple linear regression of log transformed liver Zn (Table 2-4) on dietary Zn by source indicated that the increase of Zn in liver was linear ($P<.0001$; $R^2=.78$).

The effect of dietary Zn concentration and source on mineral concentration of kidney is shown in Table 2-5. Zinc and copper concentrations in kidney were increased ($P<.0001$) by dietary Zn intake. Kidney Fe was not influenced by Zn source. Multiple linear regression of log transformed kidney Zn (Table 2-4) on dietary Zn by source indicated that the increase of Zn was linear ($P<.0001$; $R^2=.81$).

Pancreas mineral concentration is summarized in Table 2-6. Pancreas Zn increased ($P<.001$) as dietary Zn increased; however, Cu and Fe were not affected by intake of high levels of Zn. Multiple linear regression of log

TABLE 2-2. PERFORMANCE OF LAMBS FED A PRACTICAL DIET SUPPLEMENTED WITH FEED GRADE ZINC SOURCES, EXP. 2

Zinc source ^a	Added Zn	Daily Intake	Body weight	
			Initial	Final
	mg/kg	kg	kg	kg
Basal	0	1.0	37.5	39.0
Sulfate-A	700	1.0	37.5	39.6
	1400	1.0	37.5	39.0
	2100	1.0	37.5	39.5
Sulfate-B	1400	1.0	37.5	39.9
Oxide-A	1400	1.0	37.5	39.4
Oxide-B	1400	1.0	37.5	38.9
SE ^b			.6	.4
ANOVA,	P value			
Source			.996	.968

Note: Each value represents six lambs. Animals fed for 23 d.

^aBasal diet contained 64 mg/kg Zn dry basis by analysis.

^bPooled standard error.

TABLE 2-3. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL AND METALLOTHIONEIN (MT) CONCENTRATION OF LIVER IN LAMBS, EXP. 2

Zinc source ^a	Added Zn, mg/kg	Liver, mg/kg ^b			Liver MT ^c
		Zn	Cu	Fe	
Basal	0	119	175	342	39
Sulfate-A	700	573	238	256	1959
	1400	686	227	219	2285
	2100	700	224	198	2440
Sulfate-B	1400	625	177	244	2002
Oxide-A	1400	603	221	278	2004
Oxide-B	1400	539	192	229	1753
SE ^d		22.3	6.4	9.9	121.5
ANOVA,		P value			
Source		.0001	.056	.012	.0001

^aBasal diet contained 64 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cWeight basis, metallothionein (MT) concentration expressed as $\mu\text{g/g}$ fresh tissue.

^dPooled standard error.

TABLE 2-4. MULTIPLE REGRESSION OF TISSUE ZINC CONCENTRATION ON DIETARY ZINC CONCENTRATION, EXP. 2

Zinc source ^b	Tissue ^a		
	Liver	Kidney	Pancreas
	Reg. equation ^c slope $\pm$ SE (10^{-5})		
Sulfate-A	53.65 $\pm$ 5.7	74.85 $\pm$ 7.7	76.86 $\pm$ 8.8
Sulfate-B	44.13 $\pm$ 5.5	65.20 $\pm$ 7.4	67.39 $\pm$ 8.5
Oxide-A	42.65 $\pm$ 5.5	65.24 $\pm$ 7.4	73.09 $\pm$ 8.5
Oxide-B	39.74 $\pm$ 5.5	62.40 $\pm$ 7.4	59.94 $\pm$ 8.5
b ₀	2.17	2.11	2.06
R ²	.78	.81	.78
SD	.14	.19	.21
P	.0001	.0001	.0001

^aDry matter basis.

^bBasal diet contained 64 mg/kg Zn dry basis by analysis.

^cRegression equation based on log 10 transformation of tissue Zn concentration, level 2100 mg/kg Zn from the Zn sulfate-A source was not included in the regression analyses.

TABLE 2-5. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION OF KIDNEY IN LAMBS, EXP. 2

Zinc source ^a	Added Zn, mg/kg	Kidney, mg/kg ^b		
		Zn	Cu	Fe
Basal	0	95	21.9	172
Sulfate-A	700	820	40.0	187
	1400	1119	51.8	192
	2100	1235	51.7	176
Sulfate-B	1400	1089	40.7	251
Oxide-A	1400	1092	56.8	226
Oxide-B	1400	1015	53.9	167
SE ^c		60.4	2.2	9.7
ANOVA,	P value			
Source		.0001	.0001	.20

Note: Each value represents six lambs. Animals fed 23 d.

^aBasal diet contained 64 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cPooled standard error.

TABLE 2-6. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION OF PANCREAS IN LAMBS, EXP. 2

Zinc source ^a	Added Zn, mg/kg	Pancreas, mg/kg ^b		
		Zn	Cu	Fe
Basal	0	87	14.1	74.2
Sulfate-A	700	784	15.3	81.3
	1400	1075	18.3	88.9
	2100	1181	16.4	70.0
Sulfate-B	1400	1118	16.5	72.1
Oxide-A	1400	1262	17.7	77.5
Oxide-B	1400	842	13.8	68.2
SE ^c		61.0	1.1	2.7
ANOVA,	P value			
Source		.0001	.89	.41

Note: Each value represents six lambs. Animals fed 23 d.

^aBasal diet contained 64 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cPooled standard error.

transformed pancreas Zn (Table 2-4) on dietary Zn by source indicated that the increase was linear ($P < .0001$; $R^2 = .78$).

Tissue Zn uptake was related linearly to dietary Zn concentration. The linear increase in liver, kidney and pancreas Zn as dietary Zn increased allows their use as bioassay criteria with sulfate-A the standard source set at 100%. The BV values, using liver Zn uptake (Table 2-7) were 82.3, 79.5 and 74.1% for the sulfate-B, oxide-A and oxide-B sources, respectively.

When kidney Zn concentration was used as the response variable, the estimated BV values were 87.1, 87.2 and 83.4% for the sulfate-B, oxide-A and oxide-B sources, respectively (Table 2-8). The BV values based on pancreas Zn concentration (Table 2-9) were 87.7, 95.1 and 80% for the sulfate-B, oxide-A and oxide-B sources, respectively.

A relative biological availability index, calculated using the mean of the estimates from the different tissues, gave values of 85.7, 87.2 and 79.2% for the sulfate-B, oxide-A and oxide-B sources, respectively.

Discussion

Wong-Valle et al. (1989a, b) indicated that smaller particle size often results in greater bioavailability of Mn sources. However, it seems that such relationship is not valid for Zn sources. Several investigators have reported a correlation between solubility in neutral ammonium citrate

TABLE 2-7. RELATIVE BIOLOGICAL AVAILABILITY OF FEED GRADE ZINC SOURCES BASED ON MULTIPLE REGRESSION OF LIVER ZINC ON DIETARY ZINC CONCENTRATION, EXP. 2

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^d %
Sulfate-A	53.65 $\pm$ 5.7 ^a	100.0 $\pm$ - -	-
Sulfate-B	44.13 $\pm$ 5.5 ^a	82.3 $\pm$ 9.4	64 - 109
Oxide-A	42.65 $\pm$ 5.5 ^a	79.5 $\pm$ 9.3	61 - 98
Oxide-B	39.74 $\pm$ 5.5 ^a	74.1 $\pm$ 9.1	56 - 92

^aBasal diet contained 64 mg/kg Zn dry basis by analysis.

^bRegression equation based on log transformation of liver Zn, values have been multiplied by 10^{-5} , slopes in the same column with the same superscript are not significantly different ($P < .05$).

^cEstimated relative biological availability (BV) value of zinc sources determined by slope ratio methodology.

^dConfidence interval at 95% for BV value of zinc sources.

TABLE 2-8. RELATIVE BIOLOGICAL AVAILABILITY OF REAGENT GRADE ZINC SOURCES BASED ON MULTIPLE REGRESSION OF KIDNEY ZINC ON DIETARY ZINC CONCENTRATION, EXP. 2

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^d %
Sulfate-A	74.85 $\pm$ 7.7 ^a	100.0 $\pm$ --	-
Sulfate-B	65.20 $\pm$ 7.4 ^a	87.1 $\pm$ 9.3	69 - 104
Oxide-A	65.24 $\pm$ 7.4 ^a	87.2 $\pm$ 9.4	69 - 102
Oxide-B	62.40 $\pm$ 7.4 ^a	83.4 $\pm$ 9.4	65 - 102

^aBasal diet contained 44 mg/kg Zn dry basis by analysis.

^bRegression equation based on log 10 transformation of liver Zn, values have been multiplied by 10⁻⁵, slopes in the same column with the same superscript are not significantly different (P<.05).

^cEstimated relative biological availability (BV) value of zinc sources determined by slope ratio methodology.

^dConfidence interval at 95% for BV value of zinc sources.

TABLE 2-9. RELATIVE BIOLOGICAL AVAILABILITY OF REAGENT GRADE ZINC SOURCES BASED ON MULTIPLE REGRESSION OF PANCREAS ZINC ON DIETARY ZINC CONCENTRATION, EXP. 2

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^d %
Sulfate-A	76.86 $\pm$ 8.8 ^a	100.0 $\pm$ --	-
Sulfate-B	67.39 $\pm$ 8.5 ^a	87.7 $\pm$ 10.1	68 - 108
Oxide-A	73.09 $\pm$ 8.5 ^a	95.1 $\pm$ 12.2	71 - 119
Oxide-B	59.94 $\pm$ 8.5 ^a	80.0 $\pm$ 11.3	56 - 100

^aBasal diet contained 44 mg/kg Zn dry basis by analysis.

^bRegression equation based on log 10 transformation of liver Zn, values have been multiplied by 10^{-5} , slopes in the same column with the same superscript are not significantly different ($P < .05$).

^cEstimated relative biological availability (BV) value of zinc sources determined by slope ratio methodology.

^dConfidence interval at 95% for BV value of zinc sources.

and BV of Mn sources (Watson et al., 1970; Black et al., 1984a; Henry et al., 1987a) and solubility in BV of Mg sources. In the present experiments, solubility in dilute HCl tended to over estimate the value of several sources so that use of this solution to screen potential Zn sources should be approached with caution. The availability of Zn metal was under estimated by all solvents utilized.

Neutral ammonium citrate and 2% citric acid also tended to underestimate the value of FG oxide-A, while over estimating sulfate-B. It does not appear that any of the solutions tested correlated very well with relative biological availability.

Feed intake and body weight of lambs fed 2100 mg/kg Zn from $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ RG and FG sulfate-A were not decreased compared to those lambs fed the other dietary treatments in both experiments. Sheep and cattle are able to safely consume diets containing far more Zn than required to meet the minimal needs (Miller et al., 1989). The difference in tissue uptake of Zn due to the effect of Zn sources indicates that these sources were utilized differently. The RG and FG oxide sources provided less bioavailable Zn than the RG and FG sulfate sources. It is possible that the chemical form of the source plays an important role in the formation of unknown complexes inside the digestive tract which in turn limit their absorption and further metabolism (Hughes, 1984; Clydesdale, 1990).

In our study, there was no indication of Zn toxicosis because a reduction in feed intake, reduced gains were not observed as they were described by Ott et al. (1966a, b). However, high levels of dietary Zn resulted in increased liver, kidney and pancreas Zn concentrations, and a similar response has been observed with calves fed 600 mg/kg Zn (Kincaid et al., 1976b).

Although no adverse effects on performance or health were noted in our two experiments, it is evident that the normal homeostatic control mechanism which prevents large elevation of tissue Zn levels had been overcome. However, in other experiments, feeding 600 mg/kg added Zn to cows did not affect the Zn content of the tissues (Kincaid et al., 1976b). Thus, the effects of high dietary Zn may be greatly influenced by age and other physiological considerations such as stage of lactation (Miller et al., 1989). The significance of this relation to the maximal safe levels which can be tolerated is not totally clear.

The principal interactions of Zn with other metals are those with Cu and Fe. The interactions of Zn with Cu in animals include a suppression of Cu absorption (Ogiso et al., 1979; Hall et al., 1979) and interference with Cu metabolism in the liver (Bremner, 1987). Studies with radioisotopes in isolated systems have shown that a Cu-Zn antagonism occurred at the absorptive level (Van Campen and Scarfe, 1967). Several investigators have reported

inhibition of copper absorption when dietary or intraluminal Zn:Cu ratios were from 500:1 to 1000:1 (Evans et al., 1973).

The vast majority of research of this nutrient interaction has focused on the high concentrations of one metal inhibiting the absorption of the other metals present in much lower concentrations. In those experiments animals were fed purified diets and the interaction effect was evaluated by in vivo and in vitro assays (Hambidge et al., 1986). Although Zn and Cu appear to be antagonistic in biological systems the doses and ratios of Zn and Cu necessary to make this interaction practically important are debatable.

In our studies lambs were fed a practical corn-soybean meal-cotton seed meal diet, and the described Zn:Cu antagonism seemed not to occur. This is sustained by the tissue Cu uptake which did not decrease as compared to those levels reported by other researchers (Starcher, 1969), in experiments conducted with other species. The liver Cu concentration observed in lambs fed high dietary levels of Zn indicated that adequate amounts of Cu were transported from the portal blood system to the liver where Cu is distributed within several pools (Prohaska, 1990). This indicated that either the amount of Zn absorbed had been insufficient to interfere with the intermediary metabolism of Cu (Prohaska, 1990) or that MT synthesis in the small intestine was not enough to bind Cu. Saylor et al. (1980)

reported that changes in dietary Cu or Zn levels fed to sheep did not affect MT content of the intestine. The same investigators proposed that the limited capacity of sheep to synthesize MT may account for the marked susceptibility of this species to Cu toxicity.

Cousins (1979) indicated that an integral component of Zn homeostatic regulation process is the synthesis of MT. Since MT can sequester Zn entering the intestinal cells, and is sensitive to Zn status, it has been proposed that this inducible protein is involved in the homeostatic regulation of Zn. Lambs fed high levels of Zn accumulated more MT in liver but also it is possible that they excreted more Zn in feces causing no further liver Zn uptake above 1400 mg/kg Zn added to the diet. Our results confirm what has been observed by other researchers tha MT accumulation is directly related to Zn intake (Whanger, 1981a, b).

It is not established whether intestinal MT is involved physiologically in Cu absorption or transport to serosal surface (Bremner and Beattie, 1990; Prohaska, 1990). This agrees with our results in which lambs fed high dietary levels of Zn had high levels of MT and also adequate Cu concentration in liver which certainly some portion of it was bounded to MT (Bremner, 1987).

The ingestion of excess amounts of Zn has been found to induce anemia and depress tissue iron levels in chicks and rats (Blalock and Hill, 1988; Magee and Matrone, 1960), but

this interaction has received little attention in ruminants fed practical type diets. Moreover, the basis of this interaction is not completely known.

The discrepancies in the literature concerning Zn, Fe and Cu interactions may reflect 1) differences in the relative amounts and forms of these elements in the diets, 2) differences in the total composition of the diets, 3) perhaps species differences, 4) differences in design of studies, and 5) differences in the variables measured. Based on the results herein reported the BV of Zn from different RG and FG sources might have been influenced by biological factors such as age of the animals, type of diet, adequate levels of other trace elements and chemical nature of the mineral source per se. Under practical nutrition, the assessment of the relative BV of Zn sources is essential because of the cost that mineral premixes represent in feeding ruminants.

In conclusion tissue uptake of Zn increased with increasing levels of dietary Zn up to 1400 mg/kg Zn from the different sources tested. Kidney and pancreas changed most in response to elevated dietary Zn levels, followed by liver. No clear clinical and physiological signs of Zn, Cu and Fe antagonism were observed in our experiments. The tissue uptake of Zn in lambs fed high levels of Zn for a short period of time with practical diets offers a practical methodology to assess the BV of Zn sources for sheep.

Summary

Two experiments were conducted to investigate the relative biological availability of reagent grade (RG) and feed grade (FG) zinc sources for lambs when added at high levels to practical diets. In Experiment 1, forty-two crossbred wethers averaging 40 kg initially were used to determine biological availability of Zn from RG sources. Animals were assigned randomly to seven treatment groups in a completely randomized design. Prior to the experiment, lambs were group-fed a commercial corn-soybean meal-cotton seed hulls diet at approximately 800 g/hd daily and grass hay was available ad libitum. The basal diet was a corn-soybean meal-cotton seed hulls diet (44 mg/kg Zn, DM basis by analysis). The diets were supplemented with 0, 700, 1400 or 2100 mg/kg Zn from RG Zn sulfate or 1400 mg/kg Zn from Zn basic carbonate, ZnO and Zn metal, added at the expense of cornstarch. Lambs were housed in individual wooden pens with expanded metal floors. Feed intake was restricted to 1000 g/hd daily (as-fed basis) and tap water was available ad libitum. Lambs were fed the treatment diets for 23 d following a 7-d adjustment period during which all animals were fed the basal diet. At the end of the experiment, animals were killed and liver, both kidneys and pancreas were excised and frozen for subsequent mineral analyses.

Feed intake was not affected by high dietary Zn levels, and final body weight was not influenced by treatments.

Liver Zn concentration was increased ($P<.0001$) by high dietary Zn intake. However, liver Cu and Fe concentration were not affected by dietary Zn concentration. Zinc and Cu concentrations in kidney were increased ($P<.0001$) by dietary Zn intake, but Fe concentration was not affected. Pancreas Zn and Cu uptake were increased ($P<.0001$) by treatments; however, pancreas Fe was not affected by dietary Zn intake. Multiple linear regression of log transformed tissue Zn on dietary Zn concentration indicated that the increase was linear for liver ($P<.0001$; $R^2=.77$), kidney ($P<.0001$; $R^2=.92$), and for pancreas ($P<.0001$; $R^2=.87$). By means of MLR and slope ratio methodology relative biological availability values (BV) were estimated for the RG sources with the standard source $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, set at 100%. The BV values using liver Zn uptake were 103.7, 102.5 and 67.9% for the carbonate, oxide and metal sources, respectively. The BV values using kidney Zn concentration as the dependent variable were 113.8, 117.1 and 79.6% for the carbonate, oxide and metal sources, respectively. When pancreas Zn concentration was used as the variable response BV were estimated to be 101.6, 98 and 80.7% for the carbonate, oxide and metal sources, respectively. A BV index, calculated using the mean of the estimates from the different tissues, provided values of 106.4, 105.8 and 76% for the carbonate, oxide and metal sources, respectively.

In Experiment 2, forty-two crossbred wether lambs averaging 37.5 kg were allotted randomly to seven treatment groups in a completely randomized design. The basal diet was a corn-soybean meal-cotton seed hulls diet (64 mg/kg Zn DM basis by analysis). The diets were supplemented with 0, 700, 1400 or 2100 mg/kg Zn from sulfate-A, or 1400 mg/kg Zn from sulfate-B, oxide-A and oxide-B, added at the expense of cornstarch. Husbandry of lambs, tissue collection and statistical analysis were similar to those of Experiment 1. Analysis of MT concentration in liver was conducted to determine the relationship between liver Zn concentration and liver MT concentration. Feed consumption was not affected by dietary treatments; animals ate their ration within 2-3 hours. Final body weight was not decreased by high dietary Zn levels. Liver Zn and MT concentration increased ($P<.0001$) as dietary Zn increased. Liver Zn and liver MT concentration were highly correlated $r=.96$. There was a tendency to increase liver Cu ($P<.056$) as dietary Zn increased, but liver Fe decreased ($P<.012$) as the dietary Zn intake increased. Kidney Zn and Cu uptake increased ($P<.0001$) as dietary Zn increased, but kidney Fe was not affected by the treatments. Pancreas Zn was influenced ($P<.0001$) but Cu and Fe were not affected by high dietary Zn intake. Tissue Zn uptake was related linearly to dietary Zn concentration. Multiple linear regression of log transformed tissue Zn on dietary Zn by source indicated that

the increase was linear for liver ($P < .0001$; $R^2 = .78$); kidney ($P < .0001$; $R^2 = .81$) and for pancreas ($P < .0001$; $R^2 = .78$). By means of MLR and slope ratio methodology, the standard source sulfate-A set at 100%, BV were estimated for the FG sources. Using liver Zn concentration provided BV values of 82.3, 79.5 and 74.1% for sulfate-B, oxide-A and oxide-B sources, respectively. When kidney Zn was used as the response variable BV values were 87.1, 87.2 and 83.4% for sulfate-B, oxide-A and oxide-B sources, respectively. The BV based on pancreas Zn concentration was 87.7, 95.1 and 83.4% for sulfate-B, oxide-A and oxide-B, respectively. A BV index calculated using the mean of the estimates from the different tissues, gave values of 85.7, 87.2 and 79.2% for sulfate-B, oxide-A and oxide-B sources, respectively. The tissue uptake of Zn in lambs fed high dietary Zn levels for a short period of time in practical diets offers a practical methodology to assess the BV of Zn from RG and FG Zn sources for sheep.

CHAPTER 4
BIOLOGICAL AVAILABILITY OF ZINC FROM INORGANIC REAGENT AND
FEED GRADE ZINC SOURCES FED AT HIGH DIETARY LEVELS TO
BROILER CHICKS, EXPERIMENTS 3 AND 4

Introduction

Zinc was first recognized to be an essential nutrient for rats by Todd et al. (1934) and for chicks by O'Dell and Savage (1957). The necessity of Zn in animal nutrition is well established (Miller, 1970; O'Dell et al., 1958; Tucker and Salmon, 1955), but like all essential elements, Zn utilization by the animal is influenced by many factors that have not been completely described in the literature.

Recognition of the importance of Zn in avian nutrition started because of the difference in bioavailability of Zn in soybean protein and that in casein and gelatin (O'Dell and Savage, 1957). Further experiments conducted by O'Dell and Savage (1960) presented evidence that phytic acid, which is found in plant seeds, binds Zn and decreases its availability. The practical diets commonly used now consist mainly of corn and soybean meal. With these constituents the Zn content of practical diets may be limiting unless supplemented.

In most of the experiments designed to study the bioavailability of Zn, the diets employed were semipurified,

based on spray-dried egg white or soy isolate as the source of protein, and supplemented with low levels of Zn.

Edwards (1959) indicated that the Zn in Zn sulfate, Zn oxide, Zn carbonate and zinc metal was relatively available to support growth of the chick and no differences were observed in Zn availability among these sources. However, recently Wedekind and Baker (1990) reported bioavailability values for feed grade ZnO of 44.1% compared to ZnSO₄ reagent grade, set at 100% when semipurified diets were fed.

The use of semipurified diets present problems of palatability and require extra supplementation of certain nutrients such as amino acids, minerals and vitamins which in turn increase the cost of these diets. On the other hand, natural diets which are less expensive and more palatable than semipurified diets, allow the animal to perform to its genetic potential.

Several investigators have reported that young growing chickens tolerate high levels of Zn, in the form of ZnO, added to a corn-soybean meal diet without their growth or efficiency of feed utilization being affected (Mehring et al., 1956; Johnson et al., 1962; Kincaid et al., 1976a). Thus, knowing that chicks accumulate Zn in their tissues, the bioavailability of Zn from different sources could be estimated. Some investigators applying this concept have demonstrated the feasibility of using practical diets supplemented with high dietary levels of Mn and Zn for a

short period of time as a practical assay to study bioavailability of inorganic sources for poultry (Black et al., 1984a, b; Henry et al., 1987b). Tissue uptake of the element was used as the response criterion. Various methods have been reported for determining the availability of dietary mineral elements; slope ratio assay is one method to estimate bioavailability based on the regression relationship between dietary Zn level and a response of the test animal such as bone Zn (Moncilovic et al., 1975; Wedekind and Baker, 1990; Wedekind et al., 1991).

The purpose of the experiments described herein was to determine the relative biological availability (BV) of Zn for chicks of Zn from inorganic reagent grade and feed grade sources supplemented at high dietary levels to corn-soybean meal diets.

Materials and Methods

Experiment 3

This study compared the relative biological availability (BV) of Zn from reagent grade (RG) sources $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (sulfate), $3\text{Zn}(\text{OH})_2 \cdot 2\text{ZnCO}_3$ (carbonate), ZnO (oxide) and Zn metal (metal) when added at high dietary levels to practical corn-soybean meal diets. Two hundred and seventy-three, day-old feather-sexed (Ross x Ross) broiler chicks were assigned randomly to pens in thermostatically controlled, electrically heated Petersime

batteries with raised wire floors. Chicks were maintained on a 24 h constant-light cycle and allowed ad libitum access to feed and tap water.

A completely randomized design was used, with three replicate pens of seven chicks (four males and three females) assigned to each of 13 treatments. Dietary treatments included the unsupplemented basal (zero added Zn) and the basal supplemented with 400, 800 or 1200 mg/kg Zn as either sulfate, carbonate, oxide or metal.

The basal diet (Table 3-1) contained 63 mg/kg Zn (dry basis) by analysis, and was formulated to meet the requirements of the starting chick (NRC, 1984). Relative solubilities (Watson et al., 1970), magnetic susceptibility (Watson et al., 1971), chemical and physical characteristics of Zn sources were determined and X-ray diffraction patterns were interpreted (Table 1-2).

On day 20, chicks were weighed individually and feed consumption for each replicate pen determined. Chicks were killed by cervical dislocation and their liver, both kidneys and right tibia excised and frozen for subsequent analysis.

Zinc, Cu, Fe, Mn, Ca, Mg in diets and sources and Zn, Cu, and Fe in tissues were determined by flame atomic absorption spectrophotometry on a Perkin-Elmer Model 5000 with an AS-50 Autosampler (Perkin-Elmer Corporation, 1982). Standards were matched for macroelement and acid concentrations as needed and standard reference material

TABLE 3-1. COMPOSITION OF BASAL DIET, EXP. 3 AND 4

Item	%	
	Exp. 3	Exp. 4
Ingredient composition ^a		
Corn, ground yellow	55.68	55.68
Soybean meal, dehulled	37.29	37.29
Corn oil	2.50	2.50
Dicalcium phosphate	1.72	1.72
Limestone, ground	1.01	1.01
Corn starch ^b	.65	.65
Microingredients ^c	.50	.50
Salt, iodized	.40	.40
DL-methionine	.25	.25
TOTAL	100.00	100.00
Chemical composition ^d		
Dry matter, %	89.2	88.4
Crude protein, %	23.0	23.0
Metabolizable energy, kcal/kg	3016.0	3016.0
Ca, %	1.0	1.0
P, %	.95	.75
Mg, %	.21	.18
Zn, mg/kg	63	75
Cu, mg/kg	15	15
Fe, mg/kg	372	385
Mn, mg/kg	95	102

^aAs-fed basis.^bZinc supplement added at expense of corn starch.^cIngredients supplied per kilogram of diet: vitamin A palmitate, 6600 IU; vitamin D₃, 2200 ICU; menadione dimethylpyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 500 mg; vitamin B₁₂, .022 mg; ethoxyquin, 125 mg; Mn, 55 mg; Fe, 80 mg; Cu, 4 mg; Zn, 40 mg; I, .35 mg; Se, .2 mg.^dDry matter basis, crude protein and metabolizable energy calculated, dry matter and minerals determined by analysis.

from the National Institute of Standards and Technology was included with samples. Phosphorus in diets and sources was analyzed by a modified colorimetric assay (Harris and Popat, 1954).

Data were analyzed by two-way ANOVA and MLR analysis were performed using the GLM procedure of SAS (1985). The model included main effects of source, Zn levels and their interaction. In the ANOVA pen was used as the experimental unit for intake, gain, feed conversion and mineral data.

Zinc bioavailability was determined using $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ as the standard source by means of MLR and slope ratio methodology (Finney, 1978). Standard errors were calculated for each regression coefficient. Bone Zn concentration was used as the response variable to estimate BV of Zn from the tested sources. Due to difference in feed intake among treatments, BV of Zn is presented using two approaches 1) Based on MLR of bone Zn concentration (mg/kg) on dietary Zn concentration (mg/kg), and 2) Based on MLR of bone Zn concentration (mg/kg) on dietary Zn intake (mg/d).

Experiment 4

This study was conducted to estimate bioavailability of Zn from feed grade (FG) Zn sources when added at high dietary levels from ZnSO_4 -granular (sulfate-A), ZnSO_4 -spray dried (sulfate-B), ZnO (oxide-A), ZnO (oxide-B) or RG $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (sulfate-RG) which was used as the standard

source. Dietary levels of Zn used in this experiment were lower than those of Experiment 3, because of the decreased feed intake observed in birds fed the basal diet supplemented with Zn sulfate-RG.

Two hundred and eighty-eight, day-old feather-sexed (Ross x Ross) broiler chicks were assigned to a completely randomized design. Birds were divided into equal weight groups of six chicks (three males and three females) and assigned to three pens for each of the 16 treatments. Husbandry of chicks was similar to Experiment 3.

Dietary treatments included the unsupplemented basal (zero added Zn) and the basal supplemented with 300, 600 or 900 mg/kg of Zn from either $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (sulfate-RG), ZnSO_4 -granular (sulfate-A), ZnSO_4 -spray dried (sulfate-B), ZnO (oxide-A) or ZnO (oxide-B). The basal diet (Table 3-1) contained 75 mg/kg Zn (dry basis) by analysis, and was formulated to meet the requirements of the starting chick (NRC, 1984).

Relative solubilities (Watson et al., 1970), magnetic susceptibility (Watson et al., 1971), chemical and physical characteristics of FG sources were determined and X-ray diffraction patterns were interpreted (Table 2-1).

At 21 d of age, chicks were weighed individually and feed consumption for each replicate pen determined. Chicks were killed by cervical dislocation and their liver, both

kidneys, pancreas and right tibia excised and frozen for subsequent mineral analysis.

Mineral determination of diets, sources and tissues was performed as described in Experiment 3. Statistical analyses of the data were conducted by two-way ANOVA, MLR using the GLM procedure of SAS (1985) and slope ratio methodology was used to estimate BV of Zn from the different sources.

Results

Experiment 3

Chemical and physical characteristics of mineral sources were determined and are presented in Table 1-2. Analysis of mineral sources indicated Zn contents of 22.0, 58.5, 77.4 and 95.5% for sulfate, carbonate, oxide and metal sources, respectively. The four RG sources had essentially different particle size but with certain similarities between the carbonate and oxide sources. The very low magnetic susceptibility of the sources may indicate a lack of Fe content.

The sulfate, carbonate and oxide sources had practically the same solubility (>93%) in neutral ammonium citrate, 2% citric acid and .4% hydrochloric acid. The metal was the least soluble of the four sources being quite insoluble in water and with solubilities of 5.9, 10.9 and 29.5% in neutral ammonium citrate, 2% citric acid and .4% hydrochloric acid, respectively. Solubility did not appear

to be related to particle size of the source.

Interpretation of X-ray diffraction patterns indicated variation in the theoretical chemical form of the sources.

Analysis of chick performance (Table 3-2) indicated a ($P<.001$) source x level interaction for daily intake, daily gain ($P<.001$) and feed conversion ($P<.005$). Chicks fed sulfate consumed less feed, had lower gains and poorer feed conversion than other birds fed high levels of Zn from other RG sources.

Bone mineral concentration as influenced by dietary Zn concentration and source is presented in Table 3-3. Bone Zn concentration was increased by source ($P<.0128$) and dietary Zn concentration ($P<.0003$). A source x level interaction ($P<.0004$) was observed for bone Cu uptake. The effect of source on bone Cu concentration was more evident than level of Zn in the diet, however, this influence was not consistent. Bone Fe was not affected by dietary Zn concentration or source.

Liver mineral concentration is summarized in Table 3-4. Liver Zn increased ($P<.0001$) due to dietary Zn concentration but liver Cu was not decreased by dietary Zn or source. Liver Fe was influenced by a source x level interaction ($P<.0182$). In chicks fed sulfate or oxide, liver Fe decreased as dietary Zn increased, while liver Fe tended to increase in chicks fed the other two sources.

TABLE 3-2. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON FEED INTAKE, GAIN AND FEED CONVERSION IN CHICKS, EXP. 3

Zinc source ^a	Added Zn, mg/kg	Daily intake, g	Daily gain, g	Feed/gain
Subtreatment effects ^b				
Basal	0	37.5	25.1	1.50
Sulfate	400	34.5	22.6	1.53
	800	32.0	19.9	1.60
	1200	22.5	13.1	1.72
Carbonate	400	32.5	21.1	1.54
	800	37.8	26.8	1.45
	1200	36.1	24.0	1.45
Oxide	400	35.8	22.5	1.59
	800	38.6	26.7	1.45
	1200	34.6	23.6	1.47
Metal	400	35.3	23.4	1.51
	800	37.8	26.8	1.41
	1200	39.3	26.1	1.51
SE ^c		.46	.39	.01
Source effects ^d				
Basal	-	37.5	25.1	1.50
Sulfate	-	29.7	18.5	1.62
Carbonate	-	35.5	23.7	1.50
Oxide	-	36.3	24.3	1.50
Metal	-	37.5	25.4	1.48

TABLE 3-2-- Continued

Zinc source ^a	Added Zn, mg/kg	Daily intake, g	Daily gain, g	Feed/gain
Zinc levels ^c				
-	0	37.5	25.1	1.50
-	400	34.5	22.4	1.54
-	800	36.5	24.8	1.48
-	1200	33.1	21.7	1.55
ANOVA,	P value			
Source (S)	-	.001	.001	.001
Level (L)	-	.024	.005	.009
S * L	-	.001	.001	.005

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.

^bEach value represents the mean of three pens of seven chicks, fed diets for 20 d.

^cPooled standard error.

^dEach main effect mean for source represents 9 pens, except the control that represents 3 pens.

^eEach main effect mean for Zn level represents 12 pens, except the control level that represents 3 pens.

TABLE 3-3. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN BONE OF CHICKS, EXP. 3

Zinc source ^a	Added Zn, mg/kg	Bone, mg/kg ^b		
		Zn	Cu	Fe
Subtreatment effects ^c				
Basal	0	316	14.2	337
Sulfate	400	409	12.7	325
	800	435	16.4	283
	1200	501	20.3	325
Carbonate	400	422	13.8	338
	800	438	13.7	322
	1200	446	11.8	292
Oxide	400	409	13.3	321
	800	398	9.9	301
	1200	475	12.0	318
Metal	400	397	12.5	297
	800	400	10.3	330
	1200	412	10.7	306
SE ^d		4.4	.3	4.4
Source effects ^e				
Basal	-	316	14.2	337
Sulfate	-	448	16.5	311
Carbonate	-	435	13.1	317
Oxide	-	427	11.7	313
Metal	-	425	11.2	311

TABLE 3-3-- Continued

Zinc source ^a	Added Zn, mg/kg	Bone, mg/kg ^b		
		Zn	Cu	Fe
Zinc levels ^f				
-	0	316	14.2	337
-	400	409	13.1	320
-	800	418	12.6	309
-	1200	459	13.7	310
ANOVA,		P value		
Source (S)	-	.0128	.0001	.9568
Level (L)	-	.0003	.3275	.5861
S * L	-	.1237	.0004	.1397

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.

^bAsh weight basis.

^cEach value represents the mean of three pens of seven chicks, fed diets for 20 d.

^dPooled standard error.

^eEach main effect mean for source represents 9 pens, except the control that represents 3 pens.

^fEach main effect mean for Zn level represents 12 pens, except the control level that represents 3 pens.

TABLE 3-4. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN LIVER OF CHICKS, EXP. 3

Zinc source ^a	Added Zn, mg/kg	Liver, mg/kg ^b		
		Zn	Cu	Fe
Subtreatment effects ^c				
Basal	0	75.6	16.5	285
Sulfate	400	79.8	16.9	321
	800	79.2	17.1	335
	1200	82.0	16.0	256
Carbonate	400	74.8	16.0	259
	800	82.8	15.6	288
	1200	91.0	17.0	311
Oxide	400	79.6	16.7	299
	800	78.9	15.0	279
	1200	90.6	16.8	267
Metal	400	75.6	15.2	393
	800	82.8	15.2	367
	1200	91.0	15.0	408
SE ^d		.7	.2	4.9
Source effects ^e				
Basal	-	75.6	16.5	285
Sulfate	-	80.3	16.7	304
Carbonate	-	82.9	16.2	286
Oxide	-	83.0	16.2	282
Metal	-	80.6	15.1	389

TABLE 3-4-- Continued

Zinc source ^a	Added Zn, mg/kg	Liver, mg/kg ^b		
		Zn	Cu	Fe
Zinc levels ^f				
-	0	75.6	16.5	285
-	400	77.5	16.2	318
-	800	80.9	15.7	317
-	1200	86.7	16.2	311
ANOVA, P value				
Source (S)		.4125	.1227	.0001
Level (L)		.0001	.6311	.8111
S * L		.0835	.4705	.0182

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cEach value represents the mean of three pens of seven chicks, fed diets for 20 d.

^dPooled standard error.

^eEach main effect mean for source represents 9 pens, except the control that represents 3 pens.

^fEach main effect mean for Zn level represents 12 pens, except the control level that represents 3 pens.

Mineral concentration of kidney is shown in Table 3-5. A source x level interaction ($P < .0369$) was observed for kidney Zn uptake. Increasing dietary Zn increased kidney Zn in chicks fed all sources except Zn metal. Kidney Cu concentration was not influenced by dietary Zn or source. Kidney Fe concentration was affected by Zn level ($P < .012$); however, this effect was not well defined and was probably an artifact.

Multiple linear regression of tissue Zn concentration on dietary Zn concentration is summarized in Table 3-6. Evaluation of tissues with respect to Zn uptake indicated that bone Zn was the response variable that provided the greatest response ($P < .0001$; $R^2 = .62$) compared to the other tissues. The estimated BV values of Zn using bone Zn concentration on dietary Zn concentration (Table 3-7) were 77.7, 76.8 and 45.8% for carbonate, oxide and metal sources, respectively. The slope for Zn metal was different ($P < .05$) from sulfate-RG but not from the carbonate and oxide sources.

Due to the decreased feed intake observed in chicks supplemented with high dietary Zn from sulfate-RG, a MLR of tissue Zn concentration on dietary Zn intake was performed (Table 3-8). This adjustment for feed intake indicated that the relationship between bone Zn concentration and dietary Zn intake was significant ($P < .0001$; $R^2 = .48$). Using the slope ratio methodology, the estimated BV values were 57.1,

TABLE 3-5. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN KIDNEY OF CHICKS, EXP. 3

Zinc source ^a	Added Zn, mg/kg	Kidney, mg/kg ^b		
		Zn	Cu	Fe
Subtreatment effects ^c				
Basal	0	75.5	16.6	237
Sulfate	400	77.3	16.2	239
	800	75.7	17.5	197
	1200	98.6	19.3	250
Carbonate	400	83.4	17.0	214
	800	80.6	16.0	217
	1200	90.3	16.9	250
Oxide	400	79.5	18.2	224
	800	78.9	17.2	220
	1200	86.0	19.6	218
Metal	400	84.3	19.1	245
	800	80.6	18.3	228
	1200	82.6	17.0	250
SE ^d		.9	.3	4.1
Source effects ^e				
Basal	-	75.5	16.6	237
Sulfate	-	83.9	17.7	229
Carbonate	-	84.8	16.6	227
Oxide	-	81.5	18.3	221
Metal	-	82.5	18.1	250

TABLE 3-5-- Continued

Zinc source ^a	Added Zn, mg/kg	Kidney, mg/kg ^b		
		Zn	Cu	Fe
Zinc levels ^f				
-	0	75.5	16.6	237
-	400	81.1	17.6	231
-	800	78.9	17.2	216
-	1200	89.4	18.2	249
ANOVA,		P value		
Source (S)		.6225	.3316	.1052
Level (L)		.0003	.5293	.0120
S * L		.0369	.4327	.3861

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cEach value represents the mean of three pens of seven chicks, fed diets for 20 d.

^dPooled standard error.

^eEach main effect mean for source represents 9 pens, except the control that represents 3 pens.

^fEach main effect mean for Zn level represents 12 pens, except the control level that represents 3 pens.

TABLE 3-6. MULTIPLE REGRESSION OF TISSUE ZINC CONCENTRATION ON DIETARY ZINC CONCENTRATION, EXP. 3

Tissue	Regression equation ^a	R ²	SD	P
Bone	$Y = 356.22 + .115X_1 + .089X_2 + .088X_3 + .052X_4$	.62	31.7	.0001
Liver	$Y = 73.62 + .007X_1 + .013X_2 + .012X_3 + .009X_4$	.50	4.6	.0001
Kidney	$Y = 75.12 + .013X_1 + .011X_2 + .008X_3 + .007X_4$	.31	6.8	.0106

^aWhere Y equals tissue Zn mg/kg dry basis except for bone which is mg/kg ash basis, X_1 is $ZnSO_4 \cdot 7H_2O$, X_2 is $3Zn(OH)_2 \cdot 2ZnCO_3$, X_3 is ZnO and X_4 is Zn metal.

TABLE 3-7. RELATIVE BIOLOGICAL AVAILABILITY OF REAGENT GRADE ZINC SOURCES BASED ON MULTIPLE REGRESSION OF BONE ZINC ON DIETARY ZINC CONCENTRATION, EXP. 3

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^d %
Sulfate	.1150 $\pm$.017 ^a	100.0 $\pm$ -	-
Carbonate	.0893 $\pm$.017 ^{ab}	77.7 $\pm$ 13.7	50 - 104
Oxide	.0883 $\pm$.017 ^{ab}	76.8 $\pm$ 13.6	50 - 103
Metal	.0526 $\pm$.017 ^b	45.8 $\pm$ 13.0	20 - 71

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.

^bRegression equation based on measured bone Zn concentration (mg/kg) on dietary Zn concentration (mg/kg); slopes within a column lacking a common superscript letter differ ($P < .05$).

^cEstimated relative biological availability (BV) of Zn from RG sources determined by slope ratio methodology.

^dConfidence interval at 95% for BV values.

TABLE 3-8. MULTIPLE REGRESSION OF TISSUE ZINC CONCENTRATION ON DIETARY ZINC INTAKE, EXP. 3

Tissue	Regression equation ^a	R ²	SD	P
Bone	$Y = 362.55 + 3.928X_1 + 2.244X_2 + 2.122X_3 + 1.169X_4$	.48	36.8	.0001
Liver	$Y = 73.32 + .301X_1 + .363X_2 + .342X_3 + .237X_4$	.53	4.4	.0001
Kidney	$Y = 72.42 + .309X_1 + .252X_2 + .144X_3 + .133X_4$	.15	7.6	.2267

^aWhere Y equals tissue Zn mg/kg dry basis except for bone which is mg/kg ash basis, X_1 is $ZnSO_4 \cdot 7H_2O$, X_2 is $3Zn(OH)_2 \cdot 2ZnCO_3$, X_3 is ZnO and X_4 is Zn metal.

54.0 and 29.8% for carbonate, oxide and metal sources, respectively (Table 3-9). The coefficient of regression for Zn metal was significantly different ($P < .05$) from sulfate-RG but not from the carbonate and oxide sources.

Experiment 4

Chemical and physical characteristics of mineral sources were determined and are presented in Table 2-1. Analysis of mineral sources indicated Zn contents of 33.0, 32.2, 72.0 and 71.0% for sulfate-A, sulfate-B, oxide-A and oxide-B sources, respectively. The sulfate-A had greater amounts of large particles than the sulfate-B and the two Zn oxide sources had almost similar particle sizes.

The two FG sulfate sources were completely soluble in neutral ammonium citrate, 2% citric acid, .4% hydrochloric acid and water. Oxide-A and oxide-B were quite soluble in .4% hydrochloric acid but less soluble in neutral ammonium citrate and 2% citric acid and almost completely insoluble in water. Solubility did not appear to be related to particle size. All sources tested showed very low magnetic susceptibility which may indicate a lack of Fe content. Interpretation of X-ray diffraction patterns showed a high degree of purity in Zn content for the FG Zn sources under study.

Average daily intake, average daily gain and feed conversion values of chicks supplemented with the Zn sources

TABLE 3-9. RELATIVE BIOLOGICAL AVAILABILITY OF ZINC REAGENT GRADE SOURCES BASED ON MULTIPLE REGRESSION OF BONE ZINC ON DIETARY ZINC INTAKE, EXP. 3

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^d %
Sulfate	3.928 $\pm$.76 ^a	100.0 $\pm$ -	-
Carbonate	2.244 $\pm$.54 ^a	57.1 $\pm$ 12.7	32 - 82
Oxide	2.122 $\pm$.55 ^a	54.0 $\pm$ 12.4	30 - 78
Metal	1.169 $\pm$.52 ^b	29.8 $\pm$ 11.3	8 - 52

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.

^bRegression equation based on measured bone Zn concentration (mg/kg) on dietary Zn intake (mg/d); slopes within a column lacking a common superscript letter differ ($P < .05$).

^cEstimated relative biological availability (BV) of Zn from RG sources determined by slope ratio methodology.

^dConfidence interval at 95% for BV values.

TABLE 4-1. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON FEED INTAKE, GAIN AND FEED CONVERSION IN CHICKS, EXP. 4

Zinc source ^a	Added Zn, mg/kg	Daily intake, g	Daily gain, g	Feed/gain
Subtreatment effects ^b				
Basal	0	40.8	27.1	1.50
Sulfate-RG	300	38.9	25.1	1.55
	600	26.6	14.8	1.79
	900	26.1	13.7	1.90
Sulfate-A	300	37.2	23.9	1.55
	600	36.4	23.7	1.53
	900	35.1	21.0	1.67
Sulfate-B	300	31.0	18.3	1.69
	600	32.6	18.9	1.72
	900	28.2	14.7	1.91
Oxide-A	300	36.7	23.5	1.56
	600	39.8	25.5	1.56
	900	38.7	22.2	1.74
Oxide-B	300	37.1	24.5	1.51
	600	40.9	26.5	1.54
	900	40.2	27.1	1.48
SE ^c		.5	.4	.02
Source effects ^d				
Basal	-	40.8	27.1	1.50
Sulfate-RG	-	30.5	17.9	1.70
Sulfate-A	-	36.2	22.9	1.58
Sulfate-B	-	30.6	17.3	1.77
Oxide-A	-	38.4	23.7	1.62
Oxide-B	-	39.4	26.0	1.52

TABLE 4-1-- Continued

Zinc source ^a	Added Zn, mg/kg	Daily intake, g	Daily gain, g	Feed/gain
Zinc levels ^e				
-	0	40.8	27.1	1.50
-	300	36.2	23.1	1.57
-	600	35.3	21.9	1.63
-	900	33.7	19.7	1.74
ANOVA,	P value			
Source (S)		.0001	.001	.0001
Level (L)		.1666	.007	.0011
S * L		.0066	.001	.0157

^aBasal diet contained 75 mg/kg Zn dry basis by analysis.

^bEach value represents the mean of three pens of six chicks, fed experimental diets for 21 d.

^cPooled standard error.

^dEach main effect mean for source represents 9 pens, except the control source which represents 3 pens.

^eEach main effect mean for Zn level represents 15 pens, except the control level which represents 3 pens.

are given in Table 4-1. A source x level interaction was observed for daily intake ($P<.0066$), daily gain ($P<.001$), and feed conversion ($P<.0157$). Chicks supplemented with sulfate-RG and sulfate-B had the poorest performance.

Bone mineral concentration is summarized in Table 4-2. Bone Zn ($P<.0074$) and bone Cu ($P<.0065$) were influenced by a source x level interaction but bone Fe was not affected by dietary Zn concentration or source. The degree of response to dietary Zn depended upon the source fed. Liver mineral concentration as influenced by dietary Zn concentration and source is shown in Table 4-3. Accumulation of Zn and Cu in liver was not affected by dietary Zn or source. However, a source x level interaction ($P<.0156$) was observed for liver Fe concentration. Kidney Zn, Cu and Fe were not influenced by dietary Zn or source (Table 4-4).

Mineral concentration of pancreas is presented in Table 4-5. Pancreas Zn uptake was increased ($P<.0004$) by dietary Zn concentration. A source x level interaction ($P<.001$) was observed for pancreas Cu and Fe uptake but no consistent trend was observed.

Regression of tissue Zn concentration on dietary Zn concentration is summarized in Table 4-6. The estimated BV of Zn using bone Zn concentration (Table 4-7) were 99.3, 80.9, 77.6 and 53.9% for the sulfate-A, sulfate-B, oxide-A and oxide-B, respectively. The coefficient of regression estimated for Zn oxide-B was different ($P<.05$) from

TABLE 4-2. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN BONE OF CHICKS, EXP. 4

Zinc source ^a	Added Zn, mg/kg	Bone, mg/kg ^b		
		Zn	Cu	Fe
Subtreatment effects ^c				
Basal	0	270	12.0	262
Sulfate-RG	300	388	12.6	249
	600	400	17.7	359
	900	488	20.2	340
Sulfate-A	300	412	13.5	286
	600	430	13.3	290
	900	459	12.9	251
Sulfate-B	300	395	14.3	288
	600	413	16.0	267
	900	436	17.2	325
Oxide-A	300	406	12.4	283
	600	415	10.9	263
	900	415	16.4	299
Oxide-B	300	376	13.6	279
	600	370	11.1	260
	900	413	10.5	254
SE ^d		4.5	.3	6.1
Source effects ^e				
Basal	-	270	12.0	262
Sulfate-RG	-	425	16.8	316
Sulfate-A	-	434	13.2	276
Sulfate-B	-	415	15.8	293
Oxide-A	-	412	13.2	282
Oxide-B	-	386	11.7	264

TABLE 4-2-- Continued

Zinc source ^a	Added Zn, mg/kg	Bone, mg/kg ^b		
		Zn	Cu	Fe
Zinc levels ^f				
-	0	270	12.0	262
-	300	395	13.3	277
-	600	406	13.8	288
-	900	442	15.4	294
ANOVA, P value				
Source (S)	-	.0089	.0001	.1201
Level (L)	-	.0001	.0290	.5346
S * L	-	.0074	.0065	.0666

^aBasal diet contained 75 mg/kg Zn dry basis by analysis.

^bAsh weight basis.

^cEach value represents the mean of three pens of six chicks, fed experimental diets for 21 d.

^dPooled standard error.

^eEach main effect mean for source represents 9 pens, except the control source which represents 3 pens.

^fEach main effect mean for Zn level represents 15 pens, except the control level which represents 3 pens.

TABLE 4-3. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN LIVER OF CHICKS, EXP. 4

Zinc source ^a	Added Zn, mg/kg	Liver, mg/kg ^b		
		Zn	Cu	Fe
Subtreatment effects ^c				
Basal	0	77.4	19.3	341
Sulfate-RG	300	80.5	19.7	326
	600	83.9	18.9	353
	900	83.2	20.5	289
Sulfate-A	300	81.5	21.1	334
	600	75.6	19.0	353
	900	89.2	19.4	413
Sulfate-B	300	79.0	20.3	353
	600	84.5	20.5	377
	900	84.0	17.0	448
Oxide-A	300	81.0	16.6	378
	600	84.5	19.6	377
	900	77.1	18.0	296
Oxide-B	300	80.3	19.5	329
	600	79.2	19.1	326
	900	85.2	17.4	338
SE ^d		.7	.3	6.2
Source effects ^e				
Basal	-	77.4	19.3	341
Sulfate-RG	-	82.5	19.7	323
Sulfate-A	-	82.1	19.8	367
Sulfate-B	-	82.5	19.3	393
Oxide-A	-	80.9	18.1	350
Oxide-B	-	81.6	18.7	331

TABLE 4-3-- Continued

Zinc source ^a	Added Zn, mg/kg	Liver, mg/kg ^b		
		Zn	Cu	Fe
Zinc levels ^f				
-	0	77.4	19.3	341
-	300	80.5	19.4	344
-	600	81.5	19.4	357
-	900	83.7	18.5	357
ANOVA, P value				
Source (S)	-	.9587	.1916	.0119
Level (L)	-	.4003	.2278	.6243
S * L	-	.0641	.0826	.0156

^aBasal diet contained 75 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cEach value represents the mean of three pens of six chicks, fed experimental diets for 21 d.

^dPooled standard error.

^eEach main effect mean for source represents 9 pens, except the control source which represents 3 pens.

^fEach main effect mean for Zn level represents 15 pens, except the control level which represents 3 pens.

TABLE 4-4. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN KIDNEY OF CHICKS, EXP. 4

Zinc source ^a	Added Zn, mg/kg	Kidney, mg/kg ^b		
		Zn	Cu	Fe
Subtreatment effects ^c				
Basal	0	72.5	13.6	247
Sulfate-RG	300	79.4	15.9	243
	600	78.1	16.9	271
	900	82.7	20.3	264
Sulfate-A	300	80.9	17.3	252
	600	80.4	17.3	239
	900	88.0	19.1	278
Sulfate-B	300	79.4	18.3	268
	600	83.1	18.6	243
	900	82.7	19.0	253
Oxide-A	300	84.6	17.2	253
	600	77.1	18.3	236
	900	80.1	18.2	245
Oxide-B	300	75.9	17.0	258
	600	79.6	16.0	266
	900	81.5	17.9	287
SE ^d		.7	.4	5.6
Source effects ^e				
Basal	-	72.5	13.6	247
Sulfate-RG	-	80.0	17.7	259
Sulfate-A	-	83.1	17.9	256
Sulfate-B	-	81.7	18.6	255
Oxide-A	-	80.6	17.9	245
Oxide-B	-	79.0	16.9	270

TABLE 4-4-- Continued

Zinc source ^a	Added Zn, mg/kg	Kidney, mg/kg ^b		
		Zn	Cu	Fe
Zinc levels ^f				
-	0	72.5	13.6	247
-	300	80.0	17.1	255
-	600	79.7	17.4	251
-	900	83.0	18.9	265
ANOVA, P value				
Source (S)	-	.5037	.8453	.7321
Level (L)	-	.2514	.2289	.5809
S * L	-	.3828	.9708	.9213

^aBasal diet contained 75 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cEach value represents the mean of three pens of six chicks, fed experimental diets for 21 d.

^dPooled standard error.

^eEach main effect mean for source represents 9 pens, except the control source which represents 3 pens.

^fEach main effect mean for Zn level represents 15 pens, except the control level which represents 3 pens.

TABLE 4-5. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN PANCREAS OF CHICKS, EXP. 4

Zinc source ^a	Added Zn, mg/kg	Pancreas, mg/kg ^b		
		Zn	Cu	Fe
Subtreatment effects ^c				
Basal	0	79	29.7	129
Sulfate-RG	300	119	28.9	117
	600	128	46.7	172
	900	142	20.3	143
Sulfate-A	300	102	23.7	108
	600	130	26.4	119
	900	131	37.8	151
Sulfate-B	300	102	40.7	155
	600	108	37.1	114
	900	148	46.7	171
Oxide-A	300	119	33.5	120
	600	111	28.7	111
	900	128	28.2	133
Oxide-B	300	107	34.5	143
	600	115	35.3	133
	900	128	17.9	127
SE ^d		2.2	.5	2.6
Source effects ^e				
Basal	-	79	29.7	129
Sulfate-RG	-	130	37.1	144
Sulfate-A	-	121	29.3	126
Sulfate-B	-	119	41.5	147
Oxide-A	-	119	30.1	121
Oxide-B	-	117	32.0	134

TABLE 4-5-- Continued

Zinc source ^a	Added Zn, mg/kg	Pancreas, mg/kg ^b		
		Zn	Cu	Fe
Zinc levels ^f				
-	0	79	29.7	129
-	300	110	32.3	129
-	600	118	34.8	130
-	900	135	34.9	145
ANOVA, P value				
Source (S)	-	.4617	.0001	.0234
Level (L)	-	.0004	.2456	.0347
S * L	-	.4070	.0010	.0010

^aBasal diet contained 75 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cEach value represents the mean of three pens of six chicks, fed experimental diets for 21 d.

^dPooled standard error.

^eEach main effect mean for source represents 9 pens, except the control source which represents 3 pens.

^fEach main effect mean for Zn level represents 15 pens, except the control level which represents 3 pens.

TABLE 4-6. MULTIPLE REGRESSION OF TISSUE ZINC CONCENTRATION ON DIETARY ZINC CONCENTRATION, EXP. 4

Tissue	Regression equation ^a	R ²	SD	P
Bone	$Y = 335.16 + .151X_2 + .123X_3 + .118X_4 + .082X_5$	.57	35.5	.0001
Liver	$Y = 78.57 + .006X_1 + .007X_2 + .004X_3 + .002X_4 + .005X_5$	.10	5.5	.4477
Kidney	$Y = 76.86 + .006X_1 + .010X_2 + .008X_3 + .004X_4 + .003X_5$	.22	4.6	.0001
Pancreas	$Y = 90.68 + .061X_1 + .049X_2 + .052X_3 + .043X_4 + .042X_5$	.48	15.8	.0001

^aWhere Y equals tissue Zn mg/kg dry basis except for bone which is mg/kg ash basis, X_1 is $ZnSO_4 \cdot 7H_2O$ (RG), X_2 is $ZnSO_4$ -A (FG), X_3 is $ZnSO_4$ -B (FG), X_4 is ZnO-A (FG) and X_5 is ZnO-B (FG).

TABLE 4-7. RELATIVE BIOLOGICAL AVAILABILITY OF FEED GRADE ZINC SOURCES BASED ON MULTIPLE REGRESSION OF BONE ZINC ON DIETARY ZINC CONCENTRATION, EXP. 4

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^d %
Sulfate-RG	.152 $\pm$.03 ^a	100.0 $\pm$ -	-
Sulfate-A	.151 $\pm$.03 ^a	99.3 $\pm$ 18.4	63 - 135
Sulfate-B	.123 $\pm$.03 ^a	80.9 $\pm$ 17.6	46 - 115
Oxide-A	.118 $\pm$.03 ^{ab}	77.6 $\pm$ 16.4	52 - 116
Oxide-B	.082 $\pm$.03 ^b	53.9 $\pm$ 15.9	23 - 85

^aBasal diet contained 75 mg/kg Zn dry basis by analysis.

^bRegression equation based on measured bone Zn concentration (mg/kg) on dietary Zn concentration (mg/kg); slopes within a column lacking a common superscript letter differ (P<.05).

^cEstimated relative biological availability (BV) of Zn from FG sources determined by slope ratio methodology.

^dConfidence interval at 95% for BV values.

sulfate-RG, sulfate-A and sulfate-B but was not different from oxide-A.

Multiple linear regression of tissue Zn concentration on dietary Zn intake is shown in Table 4-8. Adjustment for variable feed intake provided a slightly lower R^2 value compared to that obtained with the regression equation in which bone Zn concentration was regressed on dietary Zn concentration (Table 4-6). The estimated BV values using bone Zn concentration (Table 4-9) were 77.6, 74.5, 52.2 and 37.7% for the sulfate-A, sulfate-B, oxide-A and oxide-B sources, respectively. The coefficient of regression for Zn oxide-B was different ($P < .05$) from the sulfate-RG, sulfate-A, and sulfate-B but was not different from oxide-A.

Discussion

The results indicated that ingestion of high dietary levels of Zn from RG sulfate, FG sulfate-A and FG sulfate-B were detrimental to chick performance. Previously, ingestion of excess of Zn has been observed to depress growth of rats (Magee and Matrone, 1960), quail (Hamilton et al., 1979) and chicks (Roberson and Schaible, 1960; Henry et al., 1987b). However, in our studies the effect of high dietary levels of Zn up to 1200 mg/kg Zn from sources such as oxide, metal, oxide-A and oxide-B did not appear to decrease feed intake and growth.

TABLE 4-8. MULTIPLE REGRESSION OF TISSUE ZINC CONCENTRATION ON DIETARY ZINC INTAKE, EXP. 4

Tissue	Regression equation ^a	R ²	SD	P
Bone	$Y = 335.06 + 5.46X_1 + 4.24X_2 + 4.07X_3 + 2.85X_4 + 2.06X_5$	.53	37.1	.0001
Liver	$Y = 78.09 + .260X_1 + .229X_2 + .164X_3 + .095X_4 + .165X_5$	.12	5.4	.3676
Kidney	$Y = 76.52 + .238X_1 + .319X_2 + .287X_3 + .129X_4 + .100X_5$	.24	4.6	.0338
Pancreas	$Y = 92.80 + 2.17X_1 + 1.37X_2 + 1.58X_3 + 1.04X_4 + 1.02X_5$	.39	17.3	.0007

^awhere Y equals tissue Zn mg/kg dry basis except for bone which is mg/kg ash basis, X_1 is $ZnSO_4 \cdot 7H_2O$ (RG), X_2 is $ZnSO_4$ -A (FG), X_3 is $ZnSO_4$ -B (FG), X_4 is ZnO-A (FG) and X_5 is ZnO-B (FG).

TABLE 4-9. RELATIVE BIOLOGICAL AVAILABILITY OF FEED GRADE ZINC SOURCES BASED ON MULTIPLE REGRESSION OF BONE ZINC ON DIETARY ZINC INTAKE, EXP. 4

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^d %
Sulfate-RG	5.46 $\pm$.96 ^a	100.0 $\pm$ -	-
Sulfate-A	4.24 $\pm$.73 ^a	77.6 $\pm$ 13.8	51 - 105
Sulfate-B	4.07 $\pm$.87 ^a	74.5 $\pm$ 15.0	45 - 104
Oxide-A	2.85 $\pm$.67 ^{ab}	52.2 $\pm$ 11.4	30 - 75
Oxide-B	2.06 $\pm$.64 ^b	37.7 $\pm$ 10.4	17 - 58

^aBasal diet contained 75 mg/kg Zn dry basis by analysis.

^bRegression equation based on measured bone Zn concentration (mg/kg) on dietary Zn intake (mg/d); slopes within a column lacking a common superscript letter differ ($P < .05$).

^cEstimated relative biological availability (BV) of Zn from FG sources determined by slope ratio methodology.

^dConfidence interval at 95% for BV values.

The difference observed in the performance of chicks seems to indicate that the chemical nature of the sources per se, played an important role on the absorption of the supplemental Zn (Seal and Heaton, 1983; Clydesdale, 1989). Furthermore, the difference between our studies and those reported by others (Stahl et al., 1989; Blalock and Hill, 1988) may reflect genetic differences of broiler chicks, duration of the experiments, source of Zn and type of diets.

Results of tissue uptake studies revealed that bone was the only tissue acceptable for use as a bioassay criterion for determining Zn bioavailability. Moncilovic et al. (1975) recommended total bone Zn as the measurement of choice for slope ratio assays in rats because this response best fit the prerequisites dictated by the assay technique.

In our studies BV of Zn from the tested sources was based on stored bone Zn concentration which has been reported to be available to the bird (Harland, 1975). The use of bone Zn concentration rather than total bone Zn (Wedekind and Baker, 1990) allows the equalization for a common unit of comparison due to decreased feed intake observed. The differences in BV of Zn obtained under the two approaches occurred as a consequence of the mathematical transformation that was used to adjust the response criterion. These results suggest that caution must be taken when interpreting bioavailability estimates that are

determined using slopes of regressions that have been subjected to mathematical adjustments.

Differing values for BV of a Zn source are not uncommon because there are several factors that influence bioavailability of Zn (O'Dell and Savage, 1960). It is also known that the nature of the diet will influence availability of Zn (Berg and Martinson, 1972). Thus, BV estimates obtained using semipurified diets and low levels of dietary Zn (Wedekind and Baker, 1990; Edwards, 1959) may differ from BV estimates obtained using practical type diets.

The lower BV observed for Zn oxide sources compared to Zn sulfate in the studies reported herein are in agreement with Wedekind and Baker (1990). Therefore, the chemical determination of the total Zn content and the solubility of the source do not provide sufficient information on the amount of the element that is available to meet the physiological requirement of growing chicks. It is possible that interactions in addition to mineral solubility during digestion may play a prominent role in the availability and utilization of Zn by the animal.

Basolo and Johnson (1964) indicated that coordination chemistry plays an important role in the digestion and metabolism of trace minerals, especially those groups classified as transition elements. Thus supplementation of transition metals to diets as soluble inorganic forms can be

diverted to a number of complexes of unknown biological value; thus causing major differences in Zn BV among the different sources.

The differences in tissue Zn uptake among the sources evaluated in our studies may reflect differences in endogenous Zn loss, which has been reported to increase due to homeostatic mechanisms with increasing Zn absorption (Weigand and Kirchgessner, 1980). Thus, Zn homeostatic control mechanisms prevented excess uptake of Zn in liver, kidney and pancreas when dietary Zn concentration was increased approximately 12 to 19-fold above that which chicks received in the unsupplemented basal diet.

Metallothionein, a zinc binding protein, has been implicated in the regulation of Zn homeostasis (Cousins, 1985b; Dunn et al., 1987). Zinc intake has been shown to induce intestinal MT. In our studies chicks supplemented with high dietary levels of Zn might have had high concentrations of intestinal MT which are associated with reduced Zn absorption, which suggest that this protein influenced the regulation of Zn absorption and possibly the BV of Zn from the sources under study.

The function of MT in biological systems remains in question in spite of copious research. However, the current theories regarding the physiological role of this unique metal binding protein include detoxification of certain

heavy metals such as cadmium (Cd), mercury (Hg), Zn and Cu (Karin, 1985).

The results presented in this report, suggest that Cu and Fe concentration in bone, liver, kidney and pancreas in response to ingestion of high dietary levels of Zn had no consistent effects. It is possible that the factors that limit Zn BV in corn-soybean meal diets may also prevent Zn toxicosis. The antagonism between Zn and Cu affected tissues differently from those results reported by others (Stahl et al., 1989).

In at least one tissue, pancreas, the effects of Zn on Cu appear to be cumulative. This may reflect the fact that the accumulation of both elements could be related to the concentration of MT in that tissue (McCormick, 1984; Fleet et al., 1988) as a response of intake of high dietary levels of Zn. Copper has been indicated to show stronger affinity for MT than Zn (Bremner and Beattie, 1990).

In conclusion bone Zn uptake from chicks fed high but non toxic dietary levels of Zn, may be a useful criterion for determining Zn BV from inorganic sources under practical conditions. No consistent signs of antagonism of Zn, Cu and Fe were observed due to the high dietary levels of Zn from different RG and FG sources. The biochemical mechanisms by which Zn from RG or FG sulfate affects performance of chicks still remain unclear.

Summary

Two experiments were conducted to investigate the relative biological availability (BV) of reagent grade (RG) and feed grade (FG) Zn sources supplemented at high dietary levels to broiler chicks. In Experiment 3, 273 day-old feather-sexed chicks were allotted randomly to 13 treatments which include an unsupplemented basal corn-soybean meal diet (63 mg/kg Zn) or the basal supplemented with 400, 800 or 1200 mg/kg Zn from RG sulfate (22.0% Zn), carbonate (58.5% Zn), oxide (77.4% Zn) or metal (95.5% Zn). Chicks were housed in Petersime batteries and allowed ad libitum access to feed and tap water for the 20-day experiment. Feed intake ($P < .001$), daily gain ($P < .001$) and feed conversion ($P < .005$) were affected by a source x level interaction. Chicks fed sulfate, showed the poorest performance, consumed less feed and had lower gains than those fed carbonate, oxide, metal or basal diets. Bone Zn concentration increased by source ($P < .0128$) and by dietary Zn concentration ($P < .0003$). Liver Zn was increased ($P < .0001$) by dietary Zn concentration. Liver Cu was not decreased by dietary Zn or source; however, liver Fe was influenced ($P < .0182$) by a source x level interaction. A source x level interaction ($P < .05$) was observed for kidney Zn; kidney Cu was not influenced by dietary Zn or source but kidney Fe was affected ($P < .012$) by dietary Zn concentration. Multiple linear regression analysis of bone Zn concentration with

respect to dietary Zn concentration indicated a significant ($P<.001$; $R^2=.62$) linear relationship between these two variables. Using the slope ratio technique with sulfate set at 100%, the BV values were 77.7, 76.8 and 45.8% for carbonate, oxide and metal sources, respectively. When bone Zn concentration was regressed on dietary Zn intake, the relationship between these two variables was also significant ($P<.001$; $R^2=.48$). Using slope ratio methodology, the estimated BV values were 57.1, 54.0 and 29.8% for carbonate, oxide and metal sources, respectively.

In Experiment 4, 288 day-old feather-sexed chicks were used to estimate the BV of Zn from FG sources. Chicks were allotted randomly to 16 treatments, which included an unsupplemented basal corn-soybean meal diet (75 mg/kg Zn) or the basal supplemented with 300, 600 or 900 mg/kg Zn as either RG sulfate (22.0% Zn), FG sulfate-A (33.0% Zn), FG sulfate-B (32.2% Zn), FG oxide-A (72.0% Zn) or FG oxide-B (71.0% Zn). During the 21-day experiment chicks had similar husbandry to Experiment 3. Feed intake ($P<.0066$), daily gain ($P<.001$) and feed conversion ($P<.0157$) were influenced by a source x level interaction. Birds fed RG sulfate, FG sulfate-A and FG sulfate-B showed the lowest performance compared to other treatments. Bone Zn ($P<.0074$) and Cu ($P<.0065$) were increased by a source x level interaction. Bone Fe was not influenced by dietary Zn or source but was numerically increased. Liver Zn and Cu were not influenced

by dietary Zn or source. A source x level interaction ($P<.0156$) was observed for liver Fe. Kidney Zn, Cu and Fe were not influenced by dietary Zn or source. Pancreas Zn uptake increased ($P<.0004$) as dietary Zn increased. A source x level interaction was observed for pancreas Cu ($P<.001$) and Fe ($P<.001$). Multiple linear regression analysis of bone Zn concentration on dietary Zn concentration indicated a significant ($P<.001$; $R^2=.57$) relationship between these two variables. Using slope ratio methodology with RG sulfate set at 100%, the BV were 99.3, 80.9, 77.6 and 53.9% for sulfate-A, sulfate-B, oxide-A and oxide-B sources, respectively. When bone Zn concentration was regressed on dietary Zn intake and slope ratio methodology was used; the estimated bioavailability were 77.6, 74.5, 52.2 and 37.7% for sulfate-A, sulfate-B, oxide-A and oxide-B sources, respectively.

CHAPTER 5
BIOLOGICAL AVAILABILITY OF ZINC FROM INORGANIC FEED GRADE
ZINC SOURCES FED AT LOW DIETARY LEVELS TO BROILER CHICKS,
EXPERIMENT 5

Introduction

Zinc is an essential nutrient not only of physiological but also of practical importance in poultry nutrition. Most proteins of plant seed origin, such as corn and soybeans, contain phytate, a phosphate ester which decreases Zn bioavailability. Because of low availability, the Zn content of practical diets may be limiting unless supplemented with Zn.

Most of the experiments conducted with chicks to study bioavailability of Zn have been conducted with semi-purified diets (Edwards, 1959; Hempe, 1987; Wedekind and Baker, 1990) to which low gradient levels of Zn were added. With few exceptions most research has been concerned with availability of Zn in feedstuffs, not supplemental Zn sources. Only a few experiments have been reported using practical type diets to estimate BV of other trace elements such as Mn, Cu and Se (Black et al., 1984a; Ledoux et al., 1991; Henry et al., 1988).

Bioavailability of Zn for several species has been determined using regression analysis and slope ratio

methodology. This technique is based on the regression relationship between dietary Zn level and a response in the test animals. Response variables used in slope ratio assays included growth rate (Forbes and Parker, 1977; Forbes et al., 1983; Wedekind and Baker, 1990), serum Zn concentration (Miller et al., 1981), total liver Zn (Franz et al., 1980), and total bone Zn (Moncilovic et al., 1975; Forbes et al., 1984).

Several compounds have been used by various workers as sources of Zn in poultry rations (Roberson and Schaible, 1960; Kackson et al., 1986). However, no detailed study has been carried out to estimate the bioavailability of Zn in various feed grade sources that are finding their way into the animal feed industry.

The applicability of using practical type diets supplemented with high but non toxic levels of Mn to determine its bioavailability has been demonstrated by Black et al., 1984a, b). However, in our previous experiments it was observed that high dietary levels of reagent grade or feed grade Zn sulfate decreased feed consumption in chicks. Therefore, it appears that supplementation of practical type diets with low dietary levels of Zn will not cause variable feed intake, and bioavailability of Zn from those sources could be estimated without possible differences in Zn intake.

The purpose of the present study was to determine the relative biological availability of Zn for growing chicks in two feed grade sources fed at low dietary levels in practical type diets.

Materials and Methods

Two hundred and forty, day-old feather-sexed (Ross x Ross) broiler chicks were assigned randomly to pens in thermostatically controlled, electrically heated Petersime batteries with raised wire floors. Chicks were raised on a 24 h constant-light cycle and allowed ad libitum access to feed and tap water.

A completely randomized design was used, with four replicate pens of six chicks (three males and three females) assigned to each of 10 treatments. Dietary treatments included the unsupplemented basal (zero added Zn) and the basal supplemented with 40, 80 or 120 mg/kg Zn as either ZnSO_4 -granular (Sulfate-A), ZnO -A (Oxide-A) or $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (Sulfate-RG) which was used as the standard source.

The basal diet (Table 5-1) contained 35 mg/kg Zn (dry basis) by analysis, and was formulated to meet the requirements of the starting chick (NRC, 1984). On day 20 chicks were weighed individually and feed consumption for each replicate pen determined. Chicks were killed by cervical dislocation and their liver, both kidneys and right tibia excised and frozen for subsequent analysis.

TABLE 5-1. COMPOSITION OF BASAL DIET, EXP. 5

Item	%
Ingredient composition ^a	
Corn, ground yellow	55.18
Soybean meal, dehulled	37.29
Corn oil	2.50
Dicalcium phosphate	1.72
Limestone, ground	1.01
Corn starch ^b	.65
Vitamin mix ^c	.50
Trace mineral mix ^d	.50
Salt, iodized	.40
DL-methionine	.25
TOTAL	
	100.00
Chemical composition ^e	
Dry matter, %	89.6
Crude protein, %	23.0
Metabolizable energy, kcal/kg	3016.0
Ca, %	1.1
P, %	.8
Mg, %	.19
Zn, mg/kg	35
Cu, mg/kg	21
Fe, mg/kg	499
Mn, mg/kg	112

^aAs-fed basis.^bZinc supplement added at expense of corn starch.^cIngredients supplied per kilogram of diet: vitamin A palmitate, 6600 IU; vitamin D₃, 2200 ICU; menadione dimethylpyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 500 mg; vitamin B₁₂, .022 mg; ethoxyquin, 125 mg.^dTrace elements supplied per kilogram of diet: Cu, 8 mg; Fe, 80 mg; Mn, 60 mg; Se, .15 mg.^eDry matter basis, crude protein and metabolizable energy calculated, dry matter and minerals determined by analysis.

Zinc, Cu, Fe, Mn, Ca, Mg in diets and Zn, Cu and Fe in tissues were determined by flame atomic absorption spectrophotometry on a Perkin-Elmer Model 5000 with an AS-50 Autosampler (Perkin-Elmer Corporation, 1982). Standards were matched for macroelement and acid concentrations as needed and standard reference material from the National Institute of Standards and Technology was included with samples. Phosphorus in diets and sources was determined by a modified colorimetric procedure (Harris and Popat, 1954).

Data were analyzed by two-way ANOVA and MLR were performed using the GLM procedure of SAS (1985). The model included main effects of source, Zn levels and their interaction. In the ANOVA pen was used as the experimental unit for intake, gain, feed conversion and mineral data.

Bioavailability of Zn was determined using $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (sulfate-RG) as the standard source by means of MLR and slope ratio methodology (Finney, 1978). Standard errors were calculated for each regression coefficient. Bone Zn concentration was used as the response variable to estimate BV of Zn from the tested sources.

Bioavailability of Zn is presented using two approaches
1) Based on MLR of bone Zn concentration (mg/kg) on dietary Zn concentration (mg/kg), and 2) Based on MLR of bone Zn concentration (mg/kg) on dietary Zn intake (mg/d).

Results and Discussion

Analysis of chick performance Table (5-2) indicated an effect of source on daily intake ($P<.012$) and daily gain ($P<.0183$). Chicks fed graded levels of sulfate-RG consumed less feed and had lower gains compared to the other dietary treatments. However, the reduction in gain observed was less severe than that observed in Experiments 3 and 4. Feed conversion was not affected by source or dietary Zn concentration. Despite of the low levels of dietary Zn used in this experiment compared to the previous two experiments, feed intake was affected especially in those chicks supplemented with sulfate-RG. It is possible that other factors such as physiological and chemical nature of the source decreased chick performance (O'Dell, 1983). There is considerable disagreement about the mechanism that controls feed intake in poultry. The consumption of unpalatable diets has been reported to decrease feed intake (NRC, 1987).

Mineral concentration of bone is summarized in Table 5-3. Supplementation of Zn at 120 mg/kg from all sources under study resulted in no further increase in bone Zn concentration; therefore this level was not included in the multiple linear regression analysis. Bone Zn increased due to the effect of source ($P<.0267$) and dietary Zn concentration ($P<.0121$). Bone Cu was influenced ($P<.0337$) by dietary Zn concentration. A source x level interaction ($P<.0001$) was observed for bone Fe uptake in which birds fed

TABLE 5-2. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON FEED INTAKE, GAIN AND FEED CONVERSION IN CHICKS, EXP. 5

Zinc source ^a	Added Zn, mg/kg	Daily intake, g	Daily gain, g	Feed/gain
Subtreatment effects ^b				
Basal	0	38.1	26.2	1.50
Sulfate-RG	40	35.9	23.1	1.55
	80	33.7	22.3	1.51
	120	34.1	22.2	1.54
Sulfate-A	40	37.5	23.9	1.57
	80	34.1	22.5	1.52
	120	36.9	24.6	1.50
Oxide-A	40	36.6	24.4	1.50
	80	38.1	24.9	1.53
	120	40.4	28.1	1.44
SE ^c		.5	.4	.01
Source effects ^d				
Basal	-	38.1	26.2	1.50
Sulfate-RG	-	34.6	22.5	1.53
Sulfate-A	-	36.2	23.7	1.53
Oxide-A	-	38.4	25.8	1.50

TABLE 5-2-- Continued

Zinc source ^a	Added Zn, mg/kg	Daily intake, g	Daily gain, g	Feed/gain
Zinc levels ^c				
-	0	38.1	26.2	1.50
-	40	36.7	23.8	1.54
-	80	35.3	23.2	1.52
-	120	37.1	24.9	1.50
ANOVA,		P value		
Source (S)		.0120	.0183	.2692
Level (L)		.3031	.2756	.3405
S * L		.3093	.4898	.7360

^aBasal diet contained 35 mg/kg Zn dry basis by analysis.

^bEach value represents the mean of four pens of six chicks, fed experimental diets for 20 d.

^cPooled standard error.

^dEach main effect mean for source represents 12 pens, except the control which represents 4 pens.

^eEach main effect mean for Zn level represents 12 pens, except the control level which represents 4 pens.

TABLE 5-3. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN BONE OF CHICKS, EXP. 5

Zinc source ^a	Added Zinc, mg/kg	Bone, mg/kg ^b		
		Zn	Cu	Fe
Subtreatment effects ^c				
Basal	0	274	11.7	246
Sulfate-RG	40	381	12.4	229
	80	398	11.2	302
	120	391	11.6	318
Sulfate-A	40	368	12.5	313
	80	396	13.4	303
	120	386	11.2	266
Oxide-A	40	357	12.9	293
	80	375	9.7	291
	120	380	9.6	246
SE ^d		2.7	.3	4.5
Source effects ^e				
Basal	-	274	11.7	246
Sulfate-RG	-	390	11.7	283
Sulfate-A	-	384	12.4	294
Oxide-A	-	371	10.7	276

TABLE 5-3-- Continued

Zinc source ^a	Added Zinc, mg/kg	Bone, mg/kg ^b		
		Zn	Cu	Fe
Zinc levels ^f				
-	0	274	11.7	246
-	40	369	12.6	278
-	80	390	11.4	299
-	120	386	10.8	276
ANOVA,		P value		
Source (S)	-	.0267	.0665	.2982
Level (L)	-	.0121	.0337	.1056
S * L	-	.8613	.1447	.0001

^aBasal diet contained 35 mg/kg Zn dry by analysis.

^bAsh weight basis.

^cEach value represents the mean of four pens of six chicks, fed experimental diets for 20 d.

^dPooled standard error.

^eEach main effect mean for source represents 12 pens, except the control which represents 4 pens.

^fEach main effect mean for Zn level represents 12 pens, except the control level which represents 4 pens.

greater concentrations of sulfate-RG had greater Fe concentrations and those fed other sources had lower concentrations.

Liver mineral concentration is presented in Table 5-4. Liver Zn, Cu and Fe were not affected by source or dietary Zn concentration. Accumulation of minerals in kidney is presented in Table 5-5. Kidney Zn was increased ($P<.0233$) by source. Kidney Cu and Fe were not affected by source or dietary Zn.

Pancreas mineral concentration is shown in Table 5-6. Pancreas Zn uptake increased ($P<.0008$) as dietary Zn increased. Pancreas Cu and Fe were not affected by source or dietary Zn. The concentration of Cu and Fe in tissues indicated that the antagonism of Zn:Cu or Zn:Fe did not occur in the same magnitude as indicated by others (Sanders, 1964; Stahl, 1989).

Table 5-7 shows MLR equation of tissue Zn concentration on dietary Zn concentration. Evaluation of bone Zn uptake indicated a ($P<.0001$; $R^2=.70$) response. The BV values of Zn using bone Zn concentration on dietary Zn concentration (Table 5-8) were 93.8 and 74.0% for sulfate-A and oxide-A sources, respectively. The slope for oxide-A was different ($P<.05$) from sulfate-RG and sulfate-A, respectively.

As described in previous experiments, chicks fed diets supplemented with sulfate-RG had decreased feed intake. It is possible that palatability of diets containing sulfate-RG

TABLE 5-4. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN LIVER OF CHICKS, EXP. 5

Zinc source ^a	Added Zinc, mg/kg	Liver, mg/kg ^b		
		Zn	Cu	Fe
Subtreatment effects ^c				
Basal	0	71.4	21.9	270
Sulfate-RG	40	73.7	17.8	350
	80	74.7	17.3	335
	120	80.7	17.3	353
Sulfate-A	40	75.5	14.6	360
	80	79.7	18.7	357
	120	79.1	17.1	352
Oxide-A	40	76.3	17.9	356
	80	80.3	17.0	400
	120	78.8	14.5	394
SE ^d		.7	.4	6.3
Source effects ^e				
Basal	-	71.4	21.9	270
Sulfate-RG	-	76.4	17.5	346
Sulfate-A	-	78.1	16.8	356
Oxide-A	-	78.5	16.5	384

TABLE 5-4-- Continued

Zinc source ^a	Added Zinc, mg/kg	Liver, mg/kg ^b		
		Zn	Cu	Fe
Zinc levels ^f				
-	0	71.4	21.9	270
-	40	75.2	16.8	355
-	80	78.2	17.7	364
-	120	79.5	16.4	367
ANOVA, P value				
Source (S)	-	.4812	.5106	.0742
Level (L)	-	.0641	.4065	.7666
S * L	-	.5057	.0929	.5840

^aBasal diet contained 35 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cEach value represents the mean of four pens of six chicks, fed experimental diets for 20 d.

^dPooled standard error.

^eEach main effect mean for source represents 12 pens, except the control which represents 4 pens.

^fEach main effect mean for Zn level represents 12 pens, except the control level which represents 4 pens.

TABLE 5-5. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN KIDNEY OF CHICKS, EXP. 5

Zinc source ^a	Added Zinc, mg/kg	Kidney, mg/kg ^b		
		Zn	Cu	Fe
Subtreatment effects ^c				
Basal	0	72.3	16.9	238
Sulfate-RG	40	71.5	19.1	234
	80	74.7	16.3	240
	120	75.0	16.8	233
Sulfate-A	40	74.1	14.8	228
	80	75.3	16.5	218
	120	74.6	16.6	213
Oxide-A	40	75.7	12.9	226
	80	77.0	16.6	230
	120	79.5	16.5	255
SE ^d		.5	.5	2.9
Source effects ^e				
Basal	-	72.3	16.9	238
Sulfate-RG	-	73.7	17.4	236
Sulfate-A	-	74.7	16.0	220
Oxide-A	-	77.4	15.3	237

TABLE 5-5--- Continued

Zinc source ^a	Added Zinc, mg/kg	Kidney, mg/kg ^b		
		Zn	Cu	Fe
Zinc levels ^f				
-	0	72.3	16.9	238
-	40	73.7	15.6	229
-	80	75.7	16.5	229
-	120	76.4	16.6	234
ANOVA, P value				
Source (S)	-	.0233	.3194	.0512
Level (L)	-	.1388	.7216	.8058
S * L	-	.7479	.3266	.1738

^aBasal diet contained 35 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cEach value represents the mean of four pens of six chicks, fed experimental diets for 20 d.

^dPooled standard error.

^eEach main effect mean for source represents 12 pens, except the control which represents 4 pens.

^fEach main effect mean for Zn level represents 12 pens, except the control level which represents 4 pens.

TABLE 5-6. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN PANCREAS OF CHICKS, EXP. 5

Zinc source ^a	Added Zinc, mg/kg	Pancreas, mg/kg ^b		
		Zn	Cu	Fe
Subtreatment effects ^c				
Basal	0	62.8	18.3	139
Sulfate-RG	40	89.0	18.2	129
	80	95.2	16.2	127
	120	103.3	16.8	139
Sulfate-A	40	92.0	16.2	138
	80	102.3	18.1	127
	120	103.1	18.6	121
Oxide-A	40	90.2	16.1	127
	80	100.2	18.2	133
	120	103.6	15.1	130
SE ^d		1.2	.4	2.9
Source effects ^e				
Basal	-	62.8	18.3	139
Sulfate-RG	-	95.8	17.0	132
Sulfate-A	-	99.1	17.6	129
Oxide-A	-	98.0	16.5	130

TABLE 5-6-- Continued

Zinc source ^a	Added Zinc, mg/kg	Pancreas, mg/kg ^b		
		Zn	Cu	Fe
Zinc levels ^f				
-	0	62.8	18.3	139
-	40	90.4	16.8	131
-	80	99.2	17.5	129
-	120	103.3	17.5	130
ANOVA,		P value		
Source (S)	-	.5671	.5342	.9011
Level (L)	-	.0008	.8381	.9335
S * L	-	.9068	.2875	.5828

^aBasal diet contained 35 mg/kg Zn dry basis by analysis.

^bDry matter basis.

^cEach value represents the mean of four pens of six chicks, fed experimental diets for 20 d.

^dPooled standard error.

^eEach main effect mean for source represents 12 pens, except the control which represents 4 pens.

^fEach main effect mean for Zn level represents 12 pens, except the control level which represents 4 pens.

TABLE 5-7. MULTIPLE REGRESSION OF TISSUE ZINC CONCENTRATION ON DIETARY ZINC CONCENTRATION, EXP. 5

Tissue	Regression equation ^a	R ²	SD	P
Bone ^b	$Y = 301.55 + 1.366X_1 + 1.281X_2 + 1.011X_3$	.70	24.7	.0001
Liver	$Y = 72.43 + .055X_1 + .067X_2 + .069X_3$	.27	4.4	.0096
Kidney	$Y = 72.49 + .019X_1 + .243X_2 + .059X_3$	.32	3.0	.0027
Pancreas	$Y = 75.18 + .247X_1 + .259X_2 + .268X_3$	.59	9.0	.0001

^aWhere Y equals tissue Zn mg/kg dry basis except for bone which is mg/kg ash basis, X_1 is $ZnSO_4 \cdot 7H_2O$ (RG), X_2 is $ZnSO_4$ -A (FG) and X_3 is ZnO-A (FG).
^bRegression equation for bone does not include the dietary level of 120 mg/kg Zn from all sources, because no further increase in bone Zn uptake was observed.

TABLE 5-8. RELATIVE BIOLOGICAL AVAILABILITY OF FEED GRADE ZINC SOURCES BASED ON MULTIPLE REGRESSION OF BONE ZINC ON DIETARY ZINC CONCENTRATION, EXP. 5

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^d %
Sulfate-RG	1.366 $\pm$.2 ^a	100.0 $\pm$ -	-
Sulfate-A	1.281 $\pm$.2 ^a	93.8 $\pm$ 18.2	58 - 129
Oxide-A	1.011 $\pm$.2 ^b	74.0 $\pm$ 17.0	41 - 107

^aBasal diet contained 35 mg/kg Zn dry basis by analysis.

^bRegression equation based on measured bone Zn concentration (mg/kg) on dietary Zn concentration (mg/kg); slopes within a column lacking a common superscript letter differ ($P < .05$).

^cEstimated relative biological availability (BV) of Zn from FG sources determined by slope-ratio methodology.

^dConfidence interval at 95% for BV values.

affected feed consumption. Johnson et al. (1962) indicated that tolerance to high levels of Zn in chicks was greater for oxide > carbonate > sulfate sources. Multiple linear regression of bone Zn concentration on dietary Zn intake (Table 5-9) indicated that this relationship was linear ($P < .001$; $R^2 = .68$). The estimated relative BV were 93.4 and 67.8% for the sulfate-A and oxide-A sources, respectively (Table 5-10). The slope for oxide-A was different ($P < .05$) from sulfate-RG and sulfate-A, respectively.

The low BV obtained for oxide-A in this experiment agrees with previous findings reported in Experiments 3 and 4. Thus, the supplementation of practical diets with low dietary levels of Zn seems to provide similar bioavailability values for Zn compared to those obtained at high dietary levels. It has been reported that the young chick has a functioning homeostatic mechanism (Kincaid et al., 1976a). This prevents excess accumulation of Zn in tissues such as liver, kidney and pancreas. However, the threshold for bone Zn uptake seems to be greater than that for other soft tissues. The benefit of Zn stored in bones for the animal has been reported (Harland, 1975). Nonetheless, the complete kinetic mechanism of Zn has not been fully described.

No clear antagonistic effect of dietary levels of Zn on Cu and Fe utilization was observed in this experiment based on general performance of the birds. In conclusion, bone Zn

TABLE 5-9. MULTIPLE REGRESSION OF TISSUE ZINC CONCENTRATION ON DIETARY ZINC INTAKE, EXP. 5

Tissue	Regression equation ^a	R ²	SD	P
Bone ^b	$Y = 301.63 + 39.36X_1 + 36.77X_2 + 26.68X_3$	.68	25.5	.0001
Liver	$Y = 73.00 + 1.37X_1 + 1.65X_2 + 1.56X_3$	.22	4.6	.0277
Kidney	$Y = 72.82 + .446X_1 + .548X_2 + 1.39X_3$	.29	3.1	.0054
Pancreas	$Y = 76.77 + 6.62X_1 + 7.03X_2 + 6.25X_3$	.53	9.7	.0001

^aWhere Y equals tissue Zn mg/kg dry basis except for bone which is mg/kg ash basis, X_1 is $ZnSO_4 \cdot 7H_2O$ (RG), X_2 is $ZnSO_4 \cdot A$ (FG) and X_3 is ZnO-A (FG).
^bRegression equation for bone does not include the dietary level of 120 mg/kg Zn from all sources, because no further increase in bone Zn uptake was observed.

TABLE 5-10. RELATIVE BIOLOGICAL AVAILABILITY OF FEED GRADE ZINC SOURCES BASED ON MULTIPLE REGRESSION OF BONE ZINC ON DIETARY ZINC INTAKE, EXP. 5

Zinc source ^a	Mult. Reg. ^b slope $\pm$ SE	BV ^c % $\pm$ SE	CI ^e %
Sulfate-RG	39.36 $\pm$ 6.1 ^a	100.0 $\pm$ -	-
Sulfate-A	36.77 $\pm$ 5.0 ^a	93.4 $\pm$ 18.3	58 - 129
Oxide-A	26.68 $\pm$ 5.5 ^a	67.8 $\pm$ 15.9	37 - 99

^aBasal diet contained 35 mg/kg Zn dry basis by analysis.

^bRegression equation based on measured bone Zn concentration (mg/kg) on dietary Zn intake (mg/d); slopes within a column lacking a common superscript letter differ ($P < .05$).

^cEstimated relative biological availability (BV) of Zn from FG sources determined by slope-ratio methodology.

^dConfidence interval at 95% for BV values.

uptake increased with increasing levels of dietary Zn up to 80 mg/kg. The sulfate-A was most available and oxide-A the least available. Bioavailability of the two sources was not related to solubility in any of the solvents used. The cost of the two sources should be taken into account when deciding which source should be used in practical type diets. Addition of either of the two feed grade sources above the nutritional requirements is not detrimental to growth of chicks.

Summary

An experiment was conducted to investigate the relative biological availability for chicks of Zn from feed grade Zn sources supplemented at low dietary levels to corn-soybean meal diets. Two hundred and forty, day-old feather-sexed broiler chicks were assigned randomly to 10 treatments which include an unsupplemented basal corn-soybean meal diet (35 mg/kg Zn) or the basal supplemented with 40, 80 or 120 mg/kg Zn from sulfate-RG (22.0% Zn), FG sulfate-A (33.0% Zn) or FG oxide-A (72.0% Zn). Chicks were housed in Petersime batteries and allowed ad libitum access to feed and tap water for the 20-day experiment. Feed intake ($P < .012$) and daily gain ($P < .0183$) were affected by source. Chicks fed graded levels of sulfate-RG consumed less feed and had lower gains compared to other treatments. However, feed conversion was not influenced by source or dietary Zn

concentration. Bone Zn increased due to the effect of source ($P<.0267$) and dietary Zn concentration ($P<.0121$), but no further bone Zn increase was observed above 80 mg/kg Zn from all sources under study. Bone Cu was influenced ($P<.0337$) by dietary Zn concentration. A source x level interaction ($P<.0001$) was observed for bone Fe uptake. Liver Zn, Cu and Fe uptake were not affected by source or dietary Zn. Kidney Zn was increased ($P<.0233$) by Zn source. Kidney Cu and Fe concentration were not affected by source or dietary Zn. Pancreas Zn uptake increased ($P<.0008$) as dietary Zn increased. Pancreas Cu and Fe concentrations were not affected by source or level of dietary Zn. Multiple linear regression analysis of bone Zn concentration on dietary Zn concentration indicated that this relationship was linear ($P<.0001$; $R^2=.70$). Using slope ratio methodology with sulfate-RG set at 100%, relative BV were 93.8 and 74.0% for the sulfate-A and oxide-A sources, respectively. When bone Zn concentration was regressed on dietary Zn intake the relationship between these two variables was linear ($P<.0001$; $R^2=.68$). The estimated relative BV were 93.4 and 67.8% for the sulfate-A and oxide-A sources, respectively.

CHAPTER 6
TOLERANCE OF BROILER CHICKS FOR HIGH DIETARY LEVELS OF ZINC
FROM DIFFERENT SOURCES AS INFLUENCED BY THE ROUTE OF
ADMINISTRATION, EXPERIMENTS 6 AND 7

Introduction

Poultry exhibit considerable tolerance to high intakes of Zn. Several reports have indicated that performance of broilers was not affected adversely by inclusion of Zn oxide to provide up to 1000 mg/kg Zn in the diet (Mehring et al., 1956; Johnson et al., 1962; Kincaid et al., 1976a; Berg and Martinson, 1972).

Roberson and Schaible (1960) indicated that dietary levels of Zn up to 1000 mg/kg from Zn oxide was tolerated to a greater degree than that from sulfate or carbonate. Similar responses were observed in our previous experiments with chicks fed practical diets supplemented with different inorganic sources of Zn. However, a decrease in chick performance was observed, especially in those chicks fed the standard source, reagent grade Zn sulfate.

Some of the effects of ingestion of high levels of Zn in poultry include a dramatic decrease in feed consumption and subsequent growth (Jackson, et al., 1986; Gibson et al., 1986; Williams et al., 1989). Thus, it is essential to examine if inclusion of other highly soluble commercial

sources cause similar effects as observed with sulfate when supplemented at high dietary levels to practical diets.

The objectives of the present study were two fold: 1) to examine the effect of two routes of Zn administration, diet versus oral dose (crop intubated) at high levels of Zn from different sources on tissue uptake of Zn, Cu and Fe in chicks fed practical diets, and 2) to estimate the relative biological availability value of Zn from different sources for chicks fed a high level of Zn from 2 to 3 wk of age.

Materials and Methods

Experiment 6

This experiment was conducted to compare the tolerance in the chick for a single oral dose (crop intubated) of a high level of Zn from different sources with a high dietary level of Zn. Forty-eight, day-old male broiler chicks were reared in thermostatically controlled, electrically heated Petersime batteries with raise floors. Chicks were maintained on a 24 h constant light schedule and allowed ad libitum access to feed and tap water.

Birds were fed a basal corn-soybean meal diet which contained 62 mg/kg Zn (dry basis) by analysis (Table 6-1) during the first 13 d of the study or pre-experimental period. The basal diet was formulated to meet the requirements of the starting chick (NRC, 1984).

TABLE 6-1. COMPOSITION OF BASAL DIET, EXP. 6 AND 7

Item	%	
	Exp. 6	Exp. 7
Ingredient composition ^a		
Corn, ground yellow	55.68	55.68
Soybean meal, dehulled	37.29	37.29
Corn oil	2.50	2.50
Dicalcium phosphate	1.72	1.72
Limestone, ground	1.01	1.01
Corn starch ^b	.65	.65
Microingredients ^c	.50	.50
Salt, iodized	.40	.40
DL-methionine	.25	.25
TOTAL	100.00	100.00
Chemical composition ^d		
Dry matter, %	89.2	90.4
Crude protein, %	23.0	23.0
Metabolizable energy, kcal/kg	3016.0	3016.0
Ca, %	1.0	1.0
P, %	.8	.8
Mg, %	.19	.19
Zn, mg/kg	62	63
Cu, mg/kg	15	20
Fe, mg/kg	490	454
Mn, mg/kg	105	95

^aAs-fed basis.^bZinc supplement added at expense of corn starch.

^cIngredients supplied per kilogram of diet: vitamin A palmitate, 6600 IU; vitamin D₃, 2200 ICU; menadione dimethylpyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 500 mg; vitamin B₁₂, .022 mg; ethoxyquin, 125 mg; Mn, 55 mg; Fe, 80 mg; Cu, 4 mg; Zn, 40 mg; I, .35 mg; Se, .2 mg.

^dDry matter basis, crude protein and metabolizable energy calculated, dry matter and minerals determined by analysis.

Relative solubilities (Watson et al., 1970), magnetic susceptibility (Watson et al., 1971), chemical and physical characteristics of Zn sources were determined (Table 6-2).

At 10 d of age chicks were assigned randomly from group-pens to individual pens (1 bird per pen) to allow them to acclimate to their new surroundings. Individual feed intake was recorded daily for all birds during the 4-day acclimation period. Birds which were to be crop intubated were removed from the cages once each day to accustom them to being handled for the intubation. At d 14 of age one-half of the birds (24 birds) were assigned randomly to four dietary treatments (1, 2, 3 and 4) which included the basal corn-soybean meal diet (62 mg/kg) or the basal supplemented with 1000 mg/kg Zn from $\text{RG ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (sulfate), $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2) \cdot 2\text{H}_2\text{O}$ (acetate) or ZnCl_2 (chloride) from 2 to 3 wk of age. The other half of the birds was assigned randomly to the other four treatments (5, 6, 7 and 8) which included the basal corn-soybean meal diet plus an oral dose (crop intubated) of water or the basal diet plus an oral dose of 1000 mg/L Zn from sulfate, acetate or chloride. The amount of source required to provide the equivalent of 1000 mg/kg Zn was calculated in relation to the feed intake of day 14. Immediately following the single oral dose chicks were allowed to eat the basal diet for the rest of the week (from d 14 to d 21 of the study).

Feed intake for each bird was recorded daily during the experimental period (d 14 to d 21). After 24 h post-dosing, three chicks from each treatment were killed via cervical dislocation and tissues obtained were: bone, liver, kidney, pancreas and small intestine. To obtain duodenal mucosal cells the digesta were thoroughly washed out of the digestive tract with cold saline solution before the mucosal cells were collected from the upper small intestine by scrapping the tissue with a glass slide after they were cut open longitudinally. In addition, different sections of the small intestine (duodenum, jejunum and ileum) were excised for mineral analysis. The tissues from the three birds were frozen for subsequent mineral analysis. The remaining three chicks from each treatment were killed at 168 h post oral dosing.

Chicks fed supplemental Zn in the diet were killed by cervical dislocation at 21 d of age and bone, liver and kidney were removed and frozen for subsequent mineral analysis. Mineral analyses of tissues collected were performed as indicated in Experiment 3. Pancreas was excised immediately after the chicks were killed and was preserved in 10% neutral-buffered formalin for subsequent histological evaluation. The histological evaluation of the pancreas was performed at the College of Veterinary Medicine, University of Florida. A small section, approximately 1 cm in length, of the preserved tissue was embedded in paraffin, sectioned,

mounted and stained with hematoxylin and eosin. Light microscopic examination was performed and verified by independent examiners to minimize subjectivity. Gross visual examination of the digestive tract was conducted during the necropsy procedure to examine possible damages that might have occurred in the crop, proventriculus, muscular stomach and small intestine due either to the dosing procedure or high level of Zn.

Data were analyzed by two-way and three-way ANOVA using the GLM procedure of SAS (1985). Treatment means were compared using single degree of freedom contrasts (Steel and Torrie, 1980).

Experiment 7

This experiment was conducted to study the effect of two routes of Zn administration and a high dietary level of Zn from different sources on the BV of Zn for chicks fed a practical corn-soybean meal diet. Forty-eight, day-old male broiler chicks were fed a basal corn-soybean meal diet (63 mg/kg Zn) as shown in Table 6-1. The diet was formulated to meet the requirements of the starting chick (NRC, 1984). Husbandry of the birds during the first 2 wk the study was similar to that of Experiment 6. At 2 wk of age birds were assigned randomly to nine treatments in two groups. One group of birds included treatments 1, 2, 3 and 4 and were fed the basal diet supplemented with either 0 or 1000 mg/kg

Zn from RG $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ (sulfate), $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2) \cdot 2\text{H}_2\text{O}$ (acetate) or ZnCl_2 (chloride). The second group of birds included treatments 5, 6, 7, 8 and 9 and were fed the unsupplemented basal diet and crop intubated with 0 (water) or 1000 mg/kg Zn from RG $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (sulfate), $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2) \cdot 2\text{H}_2\text{O}$ (acetate), ZnCl_2 (chloride) or FG $(\text{HOCH}_2[\text{CH}(\text{OH})]_4\text{CO}_2)_2\text{Zn}$ (gluconate). Oral dosing was performed daily at 1000 h from 2 to 3 wk of age. The 1000 mg/kg of Zn for each source was calculated based on feed intake of the previous day from birds fed their respective high Zn diet. During the experimental period, from 2 to 3 wk of age, feed and tap water were supplied ad libitum, and feed intake was measured daily for each chick.

At the end of the experimental period chicks were killed by cervical dislocation and bone, liver, kidney, pancreas and brain were removed and frozen for further mineral analysis. Mineral analyses for tissues collected were performed as indicated for Experiment 3. Histological evaluation of pancreas from two chicks of each treatment as well as gross visual examination of the digestive tracts were performed as indicated for Experiment 6.

Relative biological availability of Zn from the sources was estimated using mean ratio of bone Zn concentration; Zn sulfate was set as 100% bioavailable. Standard errors for BV of Zn and increase in bone Zn (%) were calculated using the "Delta Method" (Fuller, 1987; Miller, 1986).

Statistical analyses of the data collected were conducted by one-way and two-way ANOVA using the GLM procedure of SAS (1985). Treatment means were compared using single degree of freedom contrasts (Steel and Torrie, 1980).

Results

Experiment 6

Chemical and physical characteristics of Zn sources are summarized in Table 6-2. Analysis of sources indicated Zn contents of 29.2, 45.8 and 13.8% for acetate, chloride and gluconate sources, respectively. The three sources had different particle sizes but with certain similarities between the acetate and gluconate sources. The very low or nonexistent magnetic susceptibility of the sources may reflect a lack of Fe content. Solubility of the three sources was very high (>92%) in neutral ammonium citrate, 2% citric acid and .4% hydrochloric acid. Chloride had the greatest solubility in water among the three sources. Results (Table 6-3) showed a route of Zn administration x source interaction ($P < .0005$) for feed intake at 24 h post-treatment. Feed intake for chicks given a single oral dose of Zn was lower than that of chicks fed the high Zn diets (HZN). However, after the oral dose of Zn was stopped and birds were fed only the basal diet for the rest of the week, feed intake was no longer different among all treatments.

TABLE 6-2. CHEMICAL AND PHYSICAL CHARACTERISTICS OF ZINC SOURCES, EXP. 6 AND 7

	Zinc source		
	Acetate RG	Chloride RG	Gluconate FG
Chemical constituents, Dry matter basis			
Zn, %	29.2	45.8	13.8
Ca, %	.29	.52	.39
P, %	ND ^a	ND	ND
Mg, mg/kg	ND	.93	18.4
Cu, mg/kg	9.1	9.3	32.2
Fe, mg/kg	ND	ND	ND
Mn, mg/kg	ND	9.3	ND
Particle size, % ^b			
+ 20	18.0	3.5	14.0
+ 100	82.0	96.0	80.5
+ 200	-	.5	4.5
Physical appearance	White crystals	White crystals	White powder
Magnetic susceptibility, %	-	-	1.7
Relative solubility, % ^c			
Neutral ammonium citrate	100.0	97.5	96.5
Citric acid, 2%	96.7	100.0	94.3
HCl, .4%	100.0	97.3	92.7
Water	89.5	91.3	74.9

^aNot detected.^bRetained by a No 20 (850 μ m), 100 (150 μ m) or 200 (75 μ m) or passing through a 200 (75 μ m), respectively.^cSolubility in solvents at 37°C for 1 h with constant stirring expressed as % of total mineral content.

TABLE 6-3. EFFECTS OF SOURCE, LEVEL OF DIETARY ZINC AND ROUTE OF ZINC ADMINISTRATION ON FEED INTAKE, GAIN, AND FEED CONVERSION IN CHICKS, EXP. 6

Treat.	Zinc source ^a	Added Zn, mg/kg	FI ^b		FI ^c g	DG ^d g	FG ^e
			14	15			
Zn in the diet ^f							
1	Basal	0	50	38	64	46	1.4
2	Sulfate	1000	48	37	58	39	1.5
3	Acetate	1000	59	48	65	46	1.4
4	Chloride	1000	56	43	58	39	1.5
Zn oral dose ^g							
5	Basal	0	49	28	53	42	1.3
6	Sulfate	1000	55	8	52	45	1.4
7	Acetate	1000	60	10	52	37	1.4
8	Chloride	1000	61	24	56	37	1.5
SE ^h				1.0	1.3	.6	.02
ANOVA,			P value				
Route (R)				.0001	.1231	.4135	.8722
Source (S)				.0181	.8840	.7871	.1973
R * S				.0005	.3218	.3524	.1966
Contrast of sources							
Basal vs Others				.0301	.9107	.4592	.0785
Contrast of treatments							
1 vs 2&3&4				.1247	.2107	.3599	.9610
5 vs 6&7&8				.0004	.4213	.8110	.0331
2&3&4 vs 6&7&8				.0001	.4886	.5995	.3723
2 vs 3				.0062	.1909	.2628	.8371
6 vs 8				.0477	.4852	.5456	.2934

^aBasal diet contained 62 mg/kg Zn dry basis by analysis.

^bIntake at 14 d of age (previous to oral dosing with Zn) and at 15 d of age (24 h post-oral dosing with Zn).

^cDaily feed intake from d 15 to d 21 of the study.

^dDaily gain from d 15 to d 21 of the study.

^eFeed/gain for d 15 to d 21 of the study.

^fMean value from six chicks fed individually.

^gMean value from three chicks fed individually and crop intubated with Zn.

^hPooled standard error.

Daily gain and feed conversion calculated on d 15 through d 21 were not influenced by the route of Zn administration or source.

Results of bone Zn uptake are presented in Table 6-4. Bone Zn concentration was increased ($P<.0001$) by source at 168 h post-treatment in chicks either fed Zn in the diet (1.58 fold increase) or via a single oral dose (1.84 fold increase) compared to the basal treatments in each group. Bone Cu and Fe uptake measured at 168 h post-treatment were not influenced by the two routes of Zn administration or source.

When comparisons were made among chicks that received high levels of Zn via an oral dose and killed at 24 h or 168 h; a ($P<.0295$) source x time interaction was observed for bone Zn concentration. There was a 2.8 fold increase in bone Zn concentration at 24 h post-oral dose caused by the high dietary Zn compared to the basal treatment. Single degree of freedom contrasts indicated an increase ($P<.0001$) in bone Zn uptake for chicks fed sulfate, acetate and chloride sources compared to the basal group. Bone Cu and Fe uptake were not affected by source or post treatment time. After the oral dosing of Zn was stopped and chicks were fed the basal diet for the rest of the wk, bone Zn concentration decreased to similar levels observed for those chicks fed the basal diet supplemented with different Zn sources. Overall bone Cu and Fe concentrations were not

TABLE 6-4. EFFECTS OF SOURCE AND ROUTE OF ZINC ADMINISTRATION ON MINERAL CONCENTRATION IN BONE OF CHICKS, EXP. 6

Treat.	Zinc source ^a	Added Zn, mg/kg	Time ^b , h	Bone,mg/kg ^c		
				Zn	Cu	Fe
Zn in the diet ^d						
1	Basal	0	168	299	5.8	289
2	Sulfate	1000	168	481	11.6	283
3	Acetate	1000	168	402	9.2	342
4	Chloride	1000	168	534	8.1	357
Zn oral dose ^e						
5	Basal	0	168	266	4.4	278
6	Sulfate	1000	168	539	7.0	333
7	Acetate	1000	168	490	6.4	304
8	Chloride	1000	168	444	5.7	249
SE ^f				14.7	.8	12.3
ANOVA,			P value			
Route (R)				.6423	.1267	.3457
Source (S)				.0001	.3820	.7481
R x S				.1170	.9337	.3471
Contrast of sources						
Basal vs Others				.0001	.1349	.3539
Sulfate vs Acetate				.0895	.5424	.7217

TABLE 6-4-- Continued

Treat.	Zinc source ^a	Added Zn, mg/kg	Time ^b , h	Bone, mg/kg ^c		
				Zn	Cu	Fe
Zn oral dose ^e						
5	Basal	0	24	387	5.3	279
			168	266	4.4	278
6	Sulfate	1000	24	1236	3.0	368
			168	539	7.0	333
7	Acetate	1000	24	1054	5.9	312
			168	490	6.4	304
8	Chloride	1000	24	956	8.8	355
			168	444	5.7	249
SE ^{fg}				29.7	.8	15.2
ANOVA,			P value			
Source (S)				.0001	.6815	.5030
Time (T)				.0001	.9170	.2536
S * T				.0295	.5007	.6314
Contrast of sources						
Basal vs Others				.0001	.4770	.2566
Sulfate vs Acetate				.0626	.6322	.3622

^aBasal diet contained 62 mg/kg Zn dry basis by analysis.

^bTime post treatment at which birds were killed.

^cAsh weight basis.

^dEach value represents the mean of six chicks fed treatment diets from 2 to 3 wk of age.

^eEach value represents the mean of three chicks given a single oral dose only at 2 wk of age.

^{fg}Pooled standard error.

influenced by source or route of Zn administration.

Mineral concentration of liver is shown in Table 6-5. Liver Zn uptake measured at 168 h post-treatment was not affected by dietary Zn concentration or route of Zn administration. Chicks fed the high Zn diets and those that received a single oral dose of Zn had similar liver Zn concentration compared to the basal treatments. Liver Cu uptake was lower ($P<.0046$) in chicks dosed orally compared with those fed Zn. Liver Fe was not influenced by source or route of Zn administration. Birds that received a single oral dose of Zn had increased ($P<.0001$) liver Zn uptake at 24 but not 168 h post dosing. Liver Cu uptake was influenced ($P<.0201$) by post-treatment time being greater at 24 h post oral dose than at 168 h. When oral dosing of Zn was stopped and birds were fed the basal diet, liver Zn concentration decreased to similar levels observed for birds fed the basal diet. Liver Fe was not affected by source or post-treatment time of evaluation.

Mineral concentration of kidney is summarized in Table 6-6. Kidney Zn concentration at 168 h post-treatment was affected ($P<.0110$) by the route of Zn administration x source interaction. Birds fed Zn as sulfate had the greatest kidney Zn concentrations, while those dosed orally with the same source had the lowest concentrations. When kidney mineral uptake was analyzed for birds that received the single oral dose of Zn and killed at either 24 h or 168

TABLE 6-5. EFFECTS OF SOURCE AND ROUTE OF ZINC ADMINISTRATION ON MINERAL CONCENTRATION IN LIVER OF CHICKS, EXP. 6

Treat.	Zinc source ^a	Added Zn, mg/kg	Time ^b , h	Liver, mg/kg ^c		
				Zn	Cu	Fe
Zn in the diet ^d						
1	Basal	0	168	74.6	24.5	359
2	Sulfate	1000	168	108.2	27.6	319
3	Acetate	1000	168	101.0	39.6	405
4	Chloride	1000	168	90.3	26.6	370
Zn oral dose ^e						
5	Basal	1000	168	80.0	18.9	334
6	Sulfate	1000	168	77.7	17.4	229
7	Acetate	1000	168	88.2	18.8	378
8	Chloride	1000	168	83.8	20.3	301
SE ^f				3.7	1.8	18.8
ANOVA,			P value			
Route (R)				.1883	.0046	.2160
Source (S)				.3688	.5329	.3016
R x S				.4795	.6165	.9310

TABLE 6-5-- Continued

Treat.	Zinc source ^a	Added Zn, mg/kg	Time ^b , h	Liver, mg/kg ^c		
				Zn	Cu	Fe
Zn oral dose ^e						
5	Basal	0	24	87.5	19.8	288
			168	80.0	16.4	334
6	Sulfate	1000	24	511.0	24.9	258
			168	77.7	17.4	229
7	Acetate	1000	24	480.0	22.9	371
			168	88.2	18.8	378
8	Chloride	1000	24	384.0	21.1	257
			168	83.8	20.3	301
SE ^d				9.1	.7	25.8
ANOVA,			P value			
Source (S)				.0001	.4590	.3796
Time (T)				.0001	.0201	.7506
S * T				.0001	.5337	.9562
Contrast of sources						
Basal vs Others				.0001	.1228	.8629
Sulfate vs Chloride				.0518	.8456	.6714
Acetate vs Chloride				.0716	.9374	.2110

^aBasal diet contained 62 mg/kg Zn dry basis by analysis.

^bTime post treatment at which birds were killed.

^cDry matter basis.

^dEach value represents the mean of six chicks.

^eEach value represents the mean of three chicks.

^fPooled standard error.

TABLE 6-6. EFFECTS OF SOURCE AND ROUTE OF ZINC ADMINISTRATION ON MINERAL CONCENTRATION IN KIDNEY OF CHICKS, EXP. 6

Treat.	Zinc source ^a	Added Zn, mg/kg	Time ^b , h	Kidney, mg/kg ^c		
				Zn	Cu	Fe
Zn in the diet ^d						
1	Basal	0	168	89.9	18.5	253
2	Sulfate	1000	168	101.9	15.0	269
3	Acetate	1000	168	91.8	14.5	324
4	Chloride	1000	168	89.0	18.0	271
Zn oral dose ^e						
5	Basal	0	168	80.5	18.8	204
6	Sulfate	1000	168	68.1	16.6	206
7	Acetate	1000	168	89.5	17.4	264
8	Chloride	1000	168	82.4	17.5	239
SE				1.4	.7	9.5
ANOVA,			P value			
Route (R)				.0003	.4890	.0220
Source (S)				.5061	.4490	.1132
R x S				.0110	.8631	.9607
Contrast of treatments						
2&3&4 vs 6&7&8				.0009	.1861	.0508
2 vs 3				.0477	.8422	.1016
2 vs 4				.0130	.2049	.9639
6 vs 7				.0099	.8306	.2659

TABLE 6-6-- Continued

Treat.	Zinc source ^a	Added Zn, mg/kg	Time ^b , h	Kidney, mg/kg ^c		
				Zn	Cu	Fe
Zn oral dose ^e						
5	Basal	0	24	84.3	26.8	466
			168	80.5	18.8	204
6	Sulfate	1000	24	240.0	25.8	496
			168	68.1	16.6	206
7	Acetate	1000	24	267.0	29.4	486
			168	89.5	17.4	264
8	Chloride	1000	24	184.0	33.2	414
			168	82.4	17.5	239
SE ^g				2.5	.8	4.5
ANOVA, Source (S)			P value			
Time (T)				.0001	.3350	.0040
S * T				.0001	.0001	.0001
				.0001	.2471	.0012
Contrast of sources						
Basal vs Others				.0001	.4840	.2546
Sulfate vs Acetate				.0089	.4399	.1640
Sulfate vs Chloride				.0049	.1038	.0208
Acetate vs Chloride				.0001	.2939	.0006

^aBasal diet contained 62 mg/kg Zn dry basis by analysis.

^bTime post treatment at which birds were killed.

^cDry matter basis.

^dEach value represents the mean of six chicks.

^eEach value represents the mean of three chicks.

^fPooled standard error.

h post treatment, kidney Zn was increased ($P<.0001$) by a source x post treatment time interaction.

Contrasts indicated that all sources differed from each other. Kidney Cu increased ($P<.0001$) at 24 h post-oral dosing but then decreased to levels similar to those observed in birds fed diets containing high levels of Zn. Kidney Fe at 168 h post treatment was affected ($P<.022$) by route of Zn administration. A source x time interaction ($P<.0012$) was observed for kidney Fe in chicks given an oral dose of Zn. Higher levels of kidney Fe were observed at 24 h post-oral dosing of Zn, especially in birds fed the sulfate.

Mineral concentration of pancreas for birds that received a single oral dose of Zn is presented in Table 6-7. Pancreas Zn ($P<.0001$) and Fe ($P<.0026$) uptake increased by a source x post-treatment time interaction, while pancreas Cu was not influenced. Pancreases of those chicks that were fed high Zn diets or received a single oral dose of Zn were soft and had pinkish appearances similar to those that were fed the unsupplemented basal diet. Histological examinations did not reveal any distortion of pancreatic acinar structure and periacinar fibrotic infiltration. No abnormalities were apparent in cells of the islets of Langerghans.

Mineral concentration of the duodenal mucosal cells (IM) from chicks that received a single oral dose of Zn and

TABLE 6-7. EFFECTS OF SOURCE AND ROUTE OF ZINC ADMINISTRATION ON MINERAL CONCENTRATION IN PANCREAS OF CHICKS, EXP. 6

Treat.	Zinc source ^a	Added Zn, mg/kg	Time ^b , h	Pancreas, mg/kg ^c		
				Zn	Cu	Fe
Zn oral dose ^d						
5	Basal	0	24	153	31.3	113
			168	100	27.9	111
6	Sulfate	1000	24	766	23.2	541
			168	130	32.4	102
7	Acetate	1000	24	653	23.9	425
			168	170	39.9	113
8	Chloride	1000	24	466	34.6	255
			168	115	30.4	100
SE ^e				12.7	3.5	15.8
ANOVA,			P value			
Source (S)				.0001	.9767	.0028
Time (T)				.0001	.6706	.0001
S * T				.0001	.8069	.0026
Contrast of sources ^f						
Basal vs Others				.0001	.9672	.0018
Sulfate vs Chloride				.0015	.6737	.0117
Acetate vs chloride				.0049	.7628	.0589

^aBasal diet contained 62 mg/kg Zn dry basis by analysis.

^bTime post treatment at which birds were killed.

^cDry matter basis.

^dEach value represents the mean of three chicks.

^ePooled standard error.

^fOther contrasts not listed were not significant ($P < .05$).

killed 24 h post oral dose is summarized in Table 6-8. Zinc concentration in the IM was influenced ($P<.0026$) by a source x post-treatment time interaction, this increase was markedly elevated at 24 h post-oral dose of Zn. However, after the oral dosing was stopped and birds were fed the basal diet, Zn concentration in the IM decreased to levels similar to that observed in the control treatment. Copper uptake by the IM was increased ($P<.034$) at 24 h post-oral dose of Zn. Iron uptake by the IM was not influenced by source or post treatment-time of evaluation.

Mineral concentration of the three segments of the small intestine (duodenum, jejunum and ileum) in chicks that received a single oral dose of Zn is presented in Table 6-9. Uptake of Zn by the SI was affected ($P<.0002$) by a source x post-treatment time interaction. Zinc concentration was greater at 24 h in chicks fed sulfate or acetate than those fed chloride or the basal diet. Similarly Zn uptake was influenced ($P<.0369$) by the SI x post treatment-time interaction. Zinc uptake was higher for duodenum > jejunum > ileum especially at 24 h.

Copper uptake was influenced ($P<.0065$) by segment of the SI with duodenum and jejunum higher than ileum. Iron uptake by the SI was decreased by source ($P<.0407$), segment of the SI ($P<.0001$) and post-treatment time ($P<.0029$). Intake of high levels of Zn decreased Fe uptake especially in duodenum and jejunum. The difference in decrease of Fe

TABLE 6-8. EFFECTS OF HIGH LEVELS OF ORAL ZINC ON MINERAL CONCENTRATION IN DUODENAL MUCOSAL CELLS OF CHICKS, EXP. 6

Treat.	Zinc source ^a	Added Zn, mg/kg	Time ^b , h	IM, mg/kg ^c		
				Zn	Cu	Fe
Zn oral dose ^d						
5	Basal	0	24	92	22.1	185
			168	105	30.2	163
6	Sulfate	1000	24	669	59.1	134
			168	88	24.5	139
7	Acetate	1000	24	627	40.0	129
			168	107	25.2	180
8	Chloride	1000	24	471	46.3	153
			168	101	25.2	196
SE ^e				22.9	3.2	7.5
ANOVA,			P value			
Source (S)				.0031	.4603	.3364
Time (T)				.0001	.0340	.2339
S * T				.0026	.2007	.3120
Contrast of sources ^f						
Basal vs Others				.0005	.1803	.3084

^aBasal diet contained 62 mg/kg Zn dry basis by analysis.

^bTime post treatment at which birds were killed.

^cDry matter basis, duodenal mucosal cells (IM) each value represents the mean of three chicks.

^dChicks received an oral dose of deionized water or Zn, and were killed at 24 h and 168 h post dosing.

^ePooled standard error.

^fOther contrasts not listed were not significant ($P < .05$).

TABLE 6-9. EFFECTS OF SOURCE AND AN ORAL DOSE OF ZINC ON MINERAL CONCENTRATION IN SMALL INTESTINE OF CHICKS, EXP. 6

Zinc source ^a	Added Zn, mg/kg ^b	SI ^c	Small intestine, mg/kg ^d							
			Zn		Cu		Fe		24 h	168 h
			24 h ^e	168 h ^e	24 h	168 h	24 h	168 h		
Basal	0	D	103	92	16	20	127	105		
		J	89	85	16	19	124	147		
		I	67	69	11	14	56	78		
Sulfate	1000	D	422	110	26	18	83	90		
		J	278	76	20	11	61	135		
		I	168	60	14	8	45	57		
Acetate	1000	D	382	94	15	18	78	110		
		J	251	88	13	18	64	109		
		I	176	67	9	11	51	74		
Chloride	1000	D	202	87	21	13	80	104		
		J	201	74	17	13	74	117		
		I	155	61	15	8	70	67		
SE ^f				7.72		.88		3.59		

TABLE 6-9-- Continued

ANOVA,	P value	Small intestine		
		Zn	Cu	Fe
Source (S)	.0001		.9154	.0407
Time (T)	.0001		.0915	.0029
S x T	.0002		.1364	.5835
Small intestine (SI)	.0005		.0065	.0001
S x SI	.3218		.5226	.6101
T x SI	.0369		.7520	.1035
S x T x SI	.5003		.8761	.7593
Contrast of sources				
Basal vs Others	.0001		.7876	.0052
Sulfate vs Chloride	.0258		.6236	.5555
Acetate vs Chloride	.0391		.5283	.6995
Contrast of SI				
Duodenum vs Jejunum & Ileum	.0006		.0672	.0841
Jejunum vs Ileum	.0463		.0076	.0001

*Basal diet contained 62 mg/kg Zn dry basis by analysis.

^bZinc or deionized water was fed via a single oral dose.

^cSmall intestine where D=duodenum, J=jejunum and I=ileum.

^dConcentration of Zn, Cu and Fe as dry matter basis of small intestine.

^eTime in hours post treatment.

^fpooled standard error.

concentration in the SI as influenced by the two Zn sources was not significant.

Experiment 7

Results of chick performance are summarized in Table 7-1. When all nine treatments were analyzed by one-way ANOVA, it was observed that daily feed intake ($P<.0001$), daily gain ($P<.0001$) and feed conversion ($P<.0068$) were influenced by dietary treatments. Contrasts indicated a difference in daily intake ($P<.0005$) and daily gain ($P<.0001$) between birds fed the high Zn diets and those that received oral doses of Zn.

To compare the influence of route of Zn administration and source, a two-way ANOVA was performed with the first eight treatments. This analysis included only the basal, sulfate, acetate and chloride sources. Results indicated that daily feed intake ($P<.0251$) and daily gain ($P<.0024$) were decreased by the route of Zn administration x source interaction. Feed conversion was affected by route of Zn administration ($P<.0121$) and source ($P<.0448$).

Bone mineral concentration is presented in Table 7-2. One-way ANOVA showed an increase ($P<.0001$) in bone Zn uptake due to dietary Zn concentration. The greater increase in bone Zn concentration was observed in those chicks that received oral doses of Zn. However, bone Cu and Fe were not

TABLE 7-1. EFFECT OF DIETARY ZINC SOURCE AND ROUTE OF ZINC ADMINISTRATION ON PERFORMANCE OF CHICKS FROM TWO TO THREE WEEKS OF AGE, EXP. 7

Treat.	Zinc source ^a	Added Zn, mg/kg	DI ^b , g	DG ^c , g	Feed/gain ^d
Zn in the diet					
1	Basal	0	57.8	37.8	1.53
2	Sulfate	1000	58.1	32.8	1.77
3	Acetate	1000	57.3	35.2	1.62
4	Chloride	1000	57.8	30.7	1.88
Zn oral dose					
5	Basal	0	63.2	40.3	1.57
6	Sulfate	1000	43.0	18.2	2.36
7	Acetate	1000	38.2	18.9	2.02
8	Chloride	1000	38.3	17.2	2.23
9	Gluconate	1000	42.7	20.3	2.10
SE ^e			1.2	.8	.06
ONE-WAY ANOVA,		P value			
Treatment			.0001	.0001	.0068
Contrast of treatments					
5 vs 6&7&8&9			.0001	.0001	.0025
2&3&4 vs 6&7&8			.0005	.0001	.0016
TWO-WAY ANOVA ^f ,		P value			
Route (R)			.0004	.0001	.0121
Source (S)			.0039	.0001	.0448
R x S			.0251	.0024	.3245
Contrast of sources					
Basal vs Others			.0005	.0001	.0053
Sulfate vs Acetate			.9396	.2033	.2230
Sulfate vs Chloride			.4259	.5180	.2372
Acetate vs Chloride			.4707	.5238	.9698

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.

^bDaily intake mean from two to three weeks of age.

^cDaily gain mean from two to three weeks of age.

^dFeed/gain ratio from two to three weeks of age.

^ePooled standard error included all nine treatments.

^fANOVA included only the first eight treatments.

TABLE 7-2. EFFECT OF DIETARY ZINC SOURCES ON MINERAL CONCENTRATION IN BONE OF CHICKS, EXP. 7

			Bone, mg/kg ^b		
Treat.	Zinc source ^a	Added Zn, mg/kg	Zn	Cu	Fe
Zn in the diet ^c					
1	Basal	0	336	6.7	299
2	Sulfate	1000	481	8.5	384
3	Acetate	1000	509	5.7	364
4	Chloride	1000	487	11.9	372
Zn oral dose ^d					
5	Basal	0	375	8.9	305
6	Sulfate	1000	1621	7.9	227
7	Acetate	1000	1911	6.6	334
8	Chloride	1000	1710	7.5	346
9	Gluconate	1000	1706	9.2	277
SE ^e			25.9	1.8	15.9
ONE-WAY ANOVA,		P value			
Treatment			.0001	.6118	.3436
Contrast of treatments					
5 vs 6&7&8&9			.0001	.6758	.8671
6 vs 7			.0075	.6937	.0994
7 vs 8			.0571	.9203	.8474
7 vs 9			.0528	.4772	.3754
2&3&4 vs 6&7&8			.0001	.5227	.0773
TWO-WAY ANOVA ^f ,		P value			
Route (R)			.0001	.9497	.1660
Source (S)			.0001	.4243	.5939
R x S			.0001	.2289	.4027
Contrast of sources					
Basal vs Others			.0001	.7279	.4195
Sulfate vs Acetate			.1308	.1131	.8492
Sulfate vs Chloride			.0290	.2925	.3820

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.^bAsh weight basis.^cMean value from two to three weeks of age.^ePooled standard error included all nine treatments.^fANOVA included only the first eight treatments.

influenced by treatment diets. Two-way ANOVA showed a route of Zn administration x source interaction ($P<.0001$) for bone Zn uptake (Table 7-2).

Contrasts indicated a ($P<.0001$) difference in bone Zn uptake between chicks supplemented with the different forms of Zn compared to the control group. Contrasts indicated that bone Zn increase was greater ($P<.0290$) for sulfate than chloride. Bone Cu and Fe concentration were not influenced by route of Zn administration or source.

Relative biological availability value (BV) for Zn from the different sources was estimated under the two routes of Zn administration. Mean ratio of bone Zn concentration was used, the sulfate was assigned as the standard and set as 100% bioavailable. When bone Zn concentration of chicks fed high Zn diets was used BV values were 105.7 and 101.2% for acetate and chloride, respectively. In the same way BV were estimated for chicks that received oral doses of Zn; the BV values were 117.9, 105.5 and 105.2% for acetate, chloride and gluconate sources, respectively (Table 7-3).

Liver mineral concentration is shown in Table 7-4. One-way ANOVA indicated an increase ($P<.0001$) in liver Zn in response to dietary Zn. Liver Cu was influenced ($P<.05$) by treatment effects. Liver Fe was decreased ($P<.0001$) by intake of oral doses of Zn. Two-way ANOVA indicated a ($P<.0003$) route of Zn administration x source interaction

TABLE 7-3. EFFECT OF DIETARY ZINC SOURCES ON BONE ZINC CONCENTRATION OF CHICKS FROM TWO TO THREE WEEKS OF AGE, EXP. 7

Zinc source ^a	Added Zn, mg/kg	Bone Zn, mg/kg ^b	% Increase ^c	BV ^d
				% $\pm$ SE
Zn in the diet				
Basal	0	336 $\pm$ 25	--	--
Sulfate	1000	481 $\pm$ 14	43.2 $\pm$ 11.3	100.0 $\pm$ --
Acetate	1000	509 $\pm$ 8	51.4 $\pm$ 11.3	105.7 $\pm$ 3.6
Chloride	1000	487 $\pm$ 6	44.9 $\pm$ 10.7	101.2 $\pm$ 3.3
ONE-WAY ANOVA,		P value		
Contrast of sources				
Sulfate vs Acetate				.1748
Sulfate vs Chloride				.7697
Acetate vs Chloride				.3119
Zn oral dose				
Basal	0	375 $\pm$ 16	--	--
Sulfate	1000	1621 $\pm$ 106	332 $\pm$ 33	100.0 $\pm$ --
Acetate	1000	1911 $\pm$ 227	410 $\pm$ 31	117.9 $\pm$ 9.6
Chloride	1000	1710 $\pm$ 228	356 $\pm$ 30	105.5 $\pm$ 8.9
Gluconate	1000	1706 $\pm$ 257	355 $\pm$ 32	105.2 $\pm$ 9.4
ONE-WAY ANOVA,		P value		
Contrast of sources				
Sulfate vs Acetate				.0333
Sulfate vs Chloride				.4962
Sulfate vs Gluconate				.5147
Acetate vs Chloride				.1300
Acetate vs Gluconate				.1233

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.

^bAsh weight basis.

^cAverage bone Zn increase compared to the basal treatment.

^dRelative biological availability (BV) based on mean ratio of bone Zn concentration from acetate, chloride and gluconate sources compared to sulfate set as 100% bioavailable.

TABLE 7-4. EFFECT OF DIETARY ZINC SOURCES ON MINERAL CONCENTRATION IN LIVER OF CHICKS, EXP. 7

			Liver, mg/kg ^b		
Treat.	Zinc source ^a	Added Zn, mg/kg	Zn	Cu	Fe
Zn in the diet ^c					
1	Basal	0	88.1	23.6	418
2	Sulfate	1000	90.0	21.1	452
3	Acetate	1000	86.8	21.2	459
4	Chloride	1000	106.0	24.8	489
Zn oral dose ^d					
5	Basal	0	80.2	21.8	400
6	Sulfate	1000	306.0	27.2	175
7	Acetate	1000	392.0	20.2	174
8	Chloride	1000	330.0	22.2	240
9	Gluconate	1000	267.0	20.3	213
SE ^e			10.3	.5	18.5
ONE-WAY ANOVA,		P value			
Treatment			.0001	.0119	.0001
Contrast of treatments					
5 vs 6&7&8&9			.0001	.6722	.0027
6 vs 7			.0439	.0005	.9839
7 vs 8			.1426	.2930	.3721
7 vs 9			.0044	.9497	.5897
8 vs 9			.1348	.3223	.7214
2&3&4 vs 6&7&8			.0001	.4735	.0001
TWO-WAY ANOVA ^f ,		P value			
Route (R)			.0001	.7656	.0001
Source (S)			.0002	.1005	.3397
R x S			.0003	.0185	.1061
Contrast of sources ^g					
Basal vs Others			.0001	.7732	.1287
Sulfate vs Chloride			.1395	.0200	.9566

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.^bDry matter basis.^cMean value from two to three weeks of age.^ePooled standard error included all nine treatments.^fANOVA included only the first eight treatments.^gOther contrasts not listed were not significant (P<.05).

for liver Zn uptake. Liver Zn increased more dramatically in chicks that received oral doses of Zn than in those supplied in the diet and uptake depended upon source. Liver Cu was also influenced ($P<.0185$) by a route of Zn administration x source interaction; chicks dosed orally with sulfate had greater liver Cu concentrations than birds fed other diets. Liver Fe was decreased ($P<.0001$) in chicks given oral Zn doses compared with those fed Zn.

Mineral concentration of kidney is shown in Table 7-5. One-way ANOVA showed a ($P<.0001$) treatment effect on kidney uptake of Zn, Cu and Fe. When a two-way ANOVA was performed a route of Zn administration x source interaction was observed for kidney Zn ($P<.0001$), Cu ($P<.0248$) and Fe ($P<.0004$) concentrations. Chicks given oral Zn accumulated more Zn and Cu in kidney compared to those fed Zn in the diet. However, chicks given oral dose of Zn had lower kidney Fe than those fed Zn in the diet.

Pancreas mineral concentration is shown in Table 7-6. Analysis of all nine treatments by one-way ANOVA indicated an increase ($P<.0001$) in pancreas Zn due to dietary Zn intake. Pancreas Cu and Fe were not influenced by treatment diets. Two-way ANOVA showed a route of Zn x source interaction ($P<.0001$) for pancreas Zn concentration. Contrast of treatment means indicated that chicks given oral doses of Zn from acetate had higher pancreas Zn than those

TABLE 7-5. EFFECT OF DIETARY ZINC SOURCES ON MINERAL CONCENTRATION IN KIDNEY OF CHICKS, EXP. 7

Treat.	Zinc source ^a	Added Zn, mg/kg	Kidney, mg/kg ^b		
			Zn	Cu	Fe
Zn in the diet ^c					
1	Basal	0	80.4	18.2	208
2	Sulfate	1000	82.3	20.1	308
3	Acetate	1000	87.3	21.2	292
4	Chloride	1000	81.9	16.2	284
Zn oral dose ^d					
5	Basal	0	79.2	23.3	259
6	Sulfate	1000	159.0	28.0	202
7	Acetate	1000	189.0	35.7	204
8	Chloride	1000	177.0	34.9	246
9	Gluconate	1000	176.0	34.8	228
SE ^e			3.7	.8	5.4
ONE-WAY ANOVA,		P value			
Treatment			.0001	.0001	.0001
Contrast of treatments					
5 vs 6&7&8&9			.0001	.0010	.0398
6 vs 7			.0464	.0270	.9204
2&3&4 vs 6&7&8			.0001	.0001	.0001
TWO-WAY ANOVA ^f ,		P value			
Route (R)			.0001	.0001	.0007
Source (S)			.0001	.0179	.3615
R x S			.0001	.0248	.0004
Contrast of sources ^g					
Basal vs Others			.0001	.0102	.1332
Sulfate vs Chloride			.0415	.0546	.6800

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.^bDry matter basis.^cMean value from two to three weeks of age.^ePooled standard error included all nine treatments.^fANOVA included only the first eight treatments.^gOther contrasts not listed were not significant ($P < .05$).

TABLE 7-6. EFFECT OF DIETARY ZINC SOURCES ON MINERAL CONCENTRATION IN PANCREAS OF CHICKS, EXP. 7

Treat.	Zinc source ^a	Added Zn, mg/kg	Pancreas, mg/kg ^b		
			Zn	Cu	Fe
Zn in the diet ^c					
1	Basal	0	99	42.9	119
2	Sulfate	1000	187	42.8	234
3	Acetate	1000	211	48.1	148
4	Chloride	1000	196	45.5	133
Zn oral dose ^d					
5	Basal	0	116	56.1	140
6	Sulfate	1000	1124	38.4	149
7	Acetate	1000	1778	38.5	134
8	Chloride	1000	1593	50.5	182
9	Gluconate	1000	1335	44.9	135
SE ^e			52.2	1.9	17.6
ONE-WAY ANOVA,		P value			
Treatment			.0001	.4027	.1641
Contrast of treatments					
2&3&4 vs 6&7&8			.0001	.5138	.4539
5 vs 6&7&8&9			.0001	.0445	.7496
6 vs 7			.0030	.9827	.6712
6 vs 8			.0290	.1096	.3669
7 vs 9			.0387	.3946	.9766
TWO-WAY ANOVA ^f ,		P value			
Route (R)			.0001	.7841	.7256
Source (S)			.0001	.3301	.1879
R x S			.0001	.1792	.1430
Contrast of sources ^g					
Basal vs Others			.0001	.2318	.1906
Acetate vs Chloride			.1051	.1716	.2586

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.^bDry matter basis.^cMean value from two to three weeks of age.^ePooled standard error included all nine treatments.^fANOVA included only the first eight treatments.^gOther contrasts not listed were not significant ($P < .05$).

chicks given oral dose of Zn from sulfate and chloride. Copper and Fe uptake by pancreas were not influenced by route of Zn administration or source.

Brain Zn and Cu concentrations were not influenced by the route of Zn administration or source. Birds dosed orally with Zn had lower ($P < 0.0114$) brain Fe concentrations than birds fed Zn (Table 7-7).

Discussion

The results of the present studies indicated that the route of Zn administration can have a marked influence on performance of chicks especially on feed intake and growth. Specifically, it was observed that oral dosing of Zn resulted in the greatest depression in feed intake in the 24 h period following treatment but continued throughout the week. Fleet et al. (1988) observed similar results working with 4 wk old chicks which were given an oral dose of 16 mg of Zn. However, conclusive evidence has not been previously reported for a prolonged oral dosing of Zn from different sources.

The evaluation of tissue Zn accumulation under two different routes of Zn administration was intended to explain if the inclusion of soluble Zn sources other than sulfate may cause similar detrimental effects observed in previous experiments in order to identify a source more

TABLE 7-7. EFFECT OF DIETARY ZINC SOURCES ON MINERAL CONCENTRATION IN BRAIN OF CHICKS, EXP. 7

Treat.	Zinc source ^a	Added Zn, mg/kg	Brain, mg/kg ^b		
			Zn	Cu	Fe
Zn in the diet ^c					
1	Basal	0	65.9	21.5	107
2	Sulfate	1000	68.7	22.2	107
3	Acetate	1000	57.5	26.5	108
4	Chloride	1000	62.4	27.5	111
Zn oral dose ^d					
5	Basal	0	52.9	22.8	100
6	Sulfate	1000	66.2	25.0	105
7	Acetate	1000	70.0	25.2	91
8	Chloride	1000	63.6	18.7	95
9	Gluconate	1000	62.4	21.3	109
SE ^e			1.3	1.1	1.5
ONE-WAY ANOVA, Treatment		P value	.2093	.6820	.1379
TWO-WAY ANOVA ^f , Route (R)		P value	.4975	.5534	.0114
Source (S)			.7342	.7337	.5497
R x S			.0641	.3784	.3932

^aBasal diet contained 63 mg/kg Zn by analysis.

^bDry matter basis.

^cMean value from two to three weeks of age; three samples per treatment.

^ePooled standard error.

^fANOVA included only the first eight treatments.

suitable as a standard. Feeding of high Zn diets from 2 to 3 wk of age appeared not to cause serious deleterious effects on feed intake. In spite of the decrease in feed intake observed in chicks that received oral doses of Zn for 1 wk, the magnitude of this reduction was lower than that observed with chicks fed high Zn diets from 1 to 3 wk of age.

Bioavailability of Zn from different Zn sources supplemented at high levels to practical diets for poultry are related to the absorption mechanism of this element. It has been reported that absorption of Zn occurs through paracellular and an intracellular pathways (Cousins, 1985a).

In our studies with high dietary levels of Zn it is possible that the paracellular pathway was actively involved in the movement of Zn from the lumen to the portal blood for subsequent distributed to the tissues.

The BV values reported for acetate and chloride under the two routes of Zn administration could be considered similar. The results from our experiments indicated that Zn acetate is highly bioavailable but tended to decrease chick performance is contrary to what was reported by (Oh et al., 1979). These researchers reported no variation in body weight of chicks fed up to 4000 mg/kg Zn from acetate. Zinc gluconate is highly bioavailable and it has been suggested that it causes less stomach irritation in humans than Zn

sulfate. However, in our experiments gross examination of the digestive tract did not show any sign of lesions caused by the different sources.

Tissue Zn accumulation was markedly influenced by the route of Zn administration. The difference in magnitude of response of the same quantity of Zn by the two routes (diet vs oral dose) may be related to a difference in the rate and/or mode of Zn entry into the circulation.

It has been reported that dietary Zn intake influences MT synthesis in chickens (Oh et al., 1979). In chickens, MT accumulated to a greater extent in the pancreas than in the liver in response to Zn induction, although the synthetic rate appeared to be comparable for both tissues (McCormick, 1984). In our experiments we did not measure MT accumulation in the collected tissues. However, exposure to high levels of Zn may have led to the accumulation of Zn-MT in various tissues as indicated by Richards (1989a). In the same way when the cessation of oral dose of Zn occurred it may have resulted in a concomitant loss of tissue metal and MT-bound metal.

The regulation of Zn homeostasis at the intestinal level was also functioning as was demonstrated by the high levels of Zn found in the duodenal mucosa. It has been reported that MT in the intestinal mucosa binds Zn and works as a buffer to regulate homeostasis of this element. The

increase of Zn in the duodenal mucosa of chicks fed high levels of Zn is in agreement with similar data reported by Richards et al. (1987a).

The difference in accumulation of Zn and possibly Zn-MT in liver, kidney and pancreas of chicks fed high Zn diets compared to those that received oral doses of Zn may reflect the delay in the development of normal export pathways for Zn from the tissues. The excess intake of Zn affected Cu metabolism in different magnitudes.

No clear evidence of antagonism between Zn and Cu was observed. The increase in liver Cu following the oral dose of Zn may be related to the high affinity of Cu for MT (Bremner, 1990). Some researchers have suggested that Cu and Zn bound to MT could be available for direct ligand exchange with metalloenzymes or other metal-requiring proteins.

Excess of Zn intake especially via oral dose decreased liver Fe concentration. It is possible that more Fe was excreted by chicks consuming excess of Zn, this may suggest a decrease in Fe absorption or interference with the subsequent utilization of Fe (Stevenson et al., 1987; Storey and Greger, 1987). In our studies the levels of Fe in bone, kidney and pancreas were not affected which is contrary to the findings of Stahl et al. (1989) who fed chicks semi-purified diets and reported that excess Zn depressed Cu

concentrations in the liver and pancreas and Fe concentrations in tibia of their chicks.

In our studies brain concentration was not affected by ingestion of excess of Zn. It has been indicated that the mechanism by which Zn is taken up into the central nervous system is largely unknown. An early report by Turk (1965) reported brain Zn concentration for White Leghorns to be between 49 to 60 mg/kg dry basis. No differences were also observed in brain Zn concentration between layers fed an unsupplemented casein diet compared to those fed the casein supplemented with 60 mg/kg Zn. It seems that a highly homeostatic mechanism is in place to protect the brain against toxicosis of Zn (Crawford and Connor, 1972). It does not appear that soluble Zn concentration in brain is responsible for the observed decrease in feed intake, however, Zn may influence some other chemostatic mechanism controlling feeding behavior.

In conclusion, supplementation of high levels of Zn to corn-soybean meal diets from 2 to 3 wk of age provided a practical way to assess BV of Zn sources. In contrast to previous experiments, feed intake and growth rate generally were not affected when high dietary Zn was supplied in the diet. Administration of Zn via oral dose caused a greater decrease in feed intake and growth in chicks than dietary inclusion. It was hypothesized that an unpalatable taste

may have influenced intake of Zn sulfate but this could not be confirmed in the present experiments because the effect of the oral dose on feed intake was so detrimental. For practical purposes of estimating bioavailability of Zn sources for chicks, based on tissue Zn uptake, studies could be conducted from 2 to 3 wk of age.

Summary

Two experiments were conducted with broiler chicks and the objectives were twofold 1) to examine the effect of a high dietary level of soluble sources of Zn on tissue Zn, Cu and Fe uptake as influenced by two routes of Zn administration, and 2) to estimate the BV of Zn using bone Zn uptake as the response criterion in chicks fed high Zn diets from 2 to 3 wk of age. In Experiment 6, 48, day-old male chicks were housed in Petersime batteries and were fed a practical corn-soybean meal diet for the first 2 wk of age. At 10 d of age chicks were assigned randomly to individual pens (1 bird per pen) to allow them to acclimate to their new surroundings. Individual feed intake was recorded daily for all birds during the acclimation period (10 to 14 d). At 14 d of age, one-half of all birds (24 birds) were assigned randomly to four dietary treatments (1, 2, 3 and 4) which included the basal corn-soybean meal diet (62 mg/kg Zn) or the basal supplemented with 1000 mg/kg Zn

from RG sulfate (22.0% Zn), acetate (29.2% Zn) or chloride (45.8% Zn). The other 24 birds were assigned randomly to four treatments (5, 6, 7 and 8) which included the basal corn-soybean meal diet plus an oral dose (crop intubated) of water or the basal supplemented with an oral dose of 1000 mg/kg Zn from RG sulfate, acetate or chloride. Feed intake was recorded daily for each chick from d 14 to d 21 of the study. Chicks given an oral dose of Zn decreased ($P<.0001$) feed intake at 24 h post-oral dose compared to chicks fed either the control diet or fed high Zn diets. After the oral dose of Zn was stopped feed intake was similar for all treatments. Bone Zn evaluated at 168 h post-treatment was increased ($P<.0001$) by source, but bone Cu and Fe uptake were not affected by route of Zn administration or source. On the other hand, bone mineral uptake chicks given an oral dose of Zn showed a source x post treatment time interaction ($P<.0295$) for bone Zn concentration. This increase was larger at 24 h post-oral dose than 168 h post-oral dose. Bone Cu and Fe uptakes were not affected by source or post-treatment time. Liver Zn uptake at 168 h post treatment was not affected in chicks fed high Zn diets or in those given an oral dose of Zn; liver Cu uptake at 168 h post treatment was influenced ($P<.0046$) by route of Zn administration and liver Fe was not affected by the route of Zn administration or source. In chicks given an oral dose of Zn, liver Zn

measured at 24 h post-oral dose increased ($P<.0001$) by the effect of source x post-treatment time interaction, liver Cu increased ($P<.0201$) at 24 h post treatment but liver Fe was not affected by source or post-treatment time. A route of Zn x source interaction ($P<.011$) was observed for kidney Zn uptake at 168 h post-treatment; kidney Cu was not affected by route of Zn administration or source, and kidney Fe concentration was increased ($P<.022$) by route. Chicks given an oral dose of Zn showed an increase in kidney Zn ($P<.0001$) and Fe ($P<.0012$) due to the source x post-treatment time interaction; kidney Cu was increased ($P<.0001$) by post-treatment time being higher at 24 h than 168 h. Pancreas mineral uptake was evaluated only in chicks given an oral dose of Zn; pancreas Zn ($P<.0001$) and Fe ($P<.0026$) concentration were increased by the source x post treatment time interaction but pancreas Cu was not influenced by source or post-treatment time. Duodenal mucosal cells (IM) of chicks that received an oral dose of Zn showed an increase ($P<.0026$) in Zn uptake by the IM due to a source x post-treatment time interaction. Uptake of Cu by the IM was increased ($P<.034$) by post-treatment time. Iron uptake by the IM was not influenced by source or post-treatment time. Zinc concentration in the three segments of the small intestine was increased ($P<.001$) by the source x post-treatment time interaction. Zinc uptake was higher in

duodenum > jejunum > ileum. Copper uptake was influenced ($P<.0065$) by segment of the small intestine being higher in duodenum and jejunum than ileum. Iron uptake by the small intestine was decreased by source ($P<.0407$), segment of the small intestine ($P<.0001$) and post-treatment time ($P<.0029$). Ingestion of high levels of Zn decreased Fe uptake especially at the level of the duodenum and jejunum, respectively. In Experiment 7, 48, day-old male chicks were fed a basal corn-soybean meal diet (63 mg/kg Zn). Husbandry of the birds during the first 2 wk was similar to Experiment 6. At 2 wk of age birds were assigned randomly to nine treatments in two groups. One group of birds included treatments 1, 2, 3 and 4; chicks were fed the basal diet or the basal supplemented with 1000 mg/kg Zn from RG sulfate, acetate or chloride. The other group of birds included treatments 5, 6, 7, 8 and 9; chicks were fed the basal diet plus oral dose (crop intubated) of water or 1000 mg/kg Zn from RG sulfate, acetate, chloride or FG gluconate. The oral doses of water or Zn were administered daily at 1000 h for seven days, from 2 to 3 wk of age. Feed intake ($P<.0005$), daily gain ($P<.0001$) and feed conversion ($P<.0016$) of chicks given oral doses of Zn were affected adversely compared to those chicks fed high Zn diets. Feed intake ($P<.0251$) and daily gain ($P<.0024$) were decreased by a route of Zn administration x source interaction. Feed

conversion was decreased by the route of Zn administration ($P<.0121$) and source ($P<.0448$). Bone Zn concentration was increased ($P<.0001$) by a route of Zn administration x source interaction. The highest bone Zn uptake was observed in those chicks given oral doses of Zn compared to those fed high Zn diets. Bone Cu and Fe were not affected by route of Zn administration or source. Mean ratio of bone Zn concentration gave BV values of 105.7 and 101.2 % for RG acetate and chloride sources, respectively. Mean ratio of bone Zn concentration for chicks given oral doses of Zn provided BV values of 117.9, 105.5 and 105.2% for RG acetate, chloride and FG gluconate sources, respectively. Liver Zn concentration was increased ($P<.0001$) by a route of Zn administration x source interaction. The greatest liver Zn uptake was observed in chicks given doses of Zn. Liver Cu was influenced ($P<.0185$) by the same interaction effect. Liver Fe concentration was decreased by route of Zn administration. Chicks that received oral doses of Zn had lower ($P<.0001$) liver Fe than those fed high Zn diets. Kidney Zn ($P<.0001$), Cu ($P<.0248$) and Fe ($P<.0004$) were influenced by a route of Zn administration x source interaction. More Zn and Cu were accumulated in kidney of chicks given oral dose of Zn than those fed Zn diets. Kidney Fe was decreased in chicks dosed orally with Zn. Pancreas Zn was increased ($P<.0001$) by a route of Zn

administration x source interaction. Chicks given oral doses of Zn had the highest pancreas Zn concentration. Pancreas Cu and Fe were not affected by route of Zn administration or source. Brain Zn and Cu were not influenced by route of Zn administration or source. However, brain Fe decreased ($P < .0114$) in chicks given oral doses of Zn especially those chicks given RG acetate and chloride.

CHAPTER 7
TISSUE MINERAL UPTAKE AND ACCUMULATION OF METALLOTHIONEIN
IN LIVER AND PANCREAS OF CHICKS FED A HIGH DIETARY LEVEL
OF ZINC, EXPERIMENTS 8 AND 9

Introduction

The supplementation of broiler chick diets with up to 1200 mg/kg Zn from RG Zn sulfate has been shown to cause severe depression in food consumption and body weight. However, addition of a similar amount of Zn from other sources such as ZnO did not cause similar responses (Johnson et al., 1962).

There is a need to identify a Zn source that is highly bioavailable but does not cause decreased feed intake in birds when fed at high dietary levels of Zn. In recent years there has been an increase in the promotion and sale of several Zn supplements, especially for human consumption, but their relative biological availability value for chicks have not been determined.

Tissue uptake of Zn in chicks is related to Zn intake and it has been reported to be linear with respect to dietary levels (Henry et al., 1987b; Wedekind et al., 1991); thus utilization of Zn by the chick from different sources could be evaluated using this approach.

It is well recognized that change in Zn status of an animal results in the increased synthesis of metallothionein (MT) in many different tissues (Richards, 1987b). The four tissues which accumulate highest concentrations of MT and thus have been studied in greatest detail are the liver, kidney, intestine and pancreas. Of these four, pancreas has received the least attention despite the fact that this tissue appears to accumulate the greatest levels of MT (Oh et al., 1979). Although several research groups have examined the relationship between Zn and MT; there have been no direct comparisons of MT accumulation in liver and pancreas of chicks as influenced by high dietary level of Zn and time following the ingestion of Zn.

The objectives of the present study were two fold 1) to examine tissue Zn, Cu and Fe uptake as influenced by dietary Zn intake, and 2) to examine Zn-MT accumulation in liver and pancreas of chicks when fed high Zn diets from zero to three weeks of age.

Materials and Methods

Experiment 8

This study compared the effect of two Zn sources on performance and tissue Zn, Cu and Fe uptake in chicks fed a high dietary level of Zn. Forty, day-old, feather-sexed (Ross x Ross) female chicks were assigned randomly to pens in a thermostatically controlled electrically heated

Petersime battery with raised floors made of galvanized steel. Chicks were maintained on a 24 h constant-light cycle and allowed ad libitum access to feed and tap water.

A completely randomized design was used, with one replicate pen of eight birds for the basal treatment and two replicates of eight birds per pen for each of the two Zn sources. Treatments included the unsupplemented basal (zero added Zn) and the basal supplemented with 1000 mg/kg Zn as either RG sulfate or FG gluconate.

The basal diet shown in Table 8-1 contained 62 mg/kg Zn (dry basis) by analysis and was formulated to meet the requirements of the starting chick (NRC, 1984). Body weight of individual chicks and feed intake by pen were measured weekly. On day 21, chicks were weighed and killed by cervical dislocation. Tissues excised were: bone, liver kidney, pancreas and brain and frozen for subsequent mineral analysis. Tissue mineral analyses were conducted using atomic absorption spectrophotometry as indicated for Exp. 3.

Statistical analyses of the data were performed by one-way ANOVA using the GLM procedure of SAS (1985). Treatment means were compared using single degree of freedom contrasts (Steel and Torrie, 1980).

Experiment 9

This study was conducted to examine the accumulation of Zn-MT in both liver and pancreas of chicks

TABLE 8-1. COMPOSITION OF BASAL DIET, EXP. 8 AND 9

Item	%	
	Exp. 8	Exp. 9
Ingredient composition ^a		
Corn, ground yellow	55.68	55.68
Soybean meal, dehulled	37.29	37.29
Corn oil	2.50	2.50
Dicalcium phosphate	1.72	1.72
Limestone, ground	1.01	1.01
Corn starch ^b	.65	.65
Microingredients ^c	.50	.50
Salt, iodized	.40	.40
DL-methionine	.25	.25
TOTAL	100.00	100.00
Chemical composition ^d		
Dry matter, %	89.2	90.4
Crude protein, %	23.0	23.0
Metabolizable energy, kcal/kg	3016.0	3016.0
Ca, %	1.0	1.0
P, %	.8	.8
Mg, %	.19	.19
Zn, mg/kg	62	63
Cu, mg/kg	15	20
Fe, mg/kg	490	454
Mn, mg/kg	105	95

^aAs-fed basis.^bZinc supplement added at expense of corn starch.

^cIngredients supplied per kilogram of diet: vitamin A palmitate, 6600 IU; vitamin D₃, 2200 ICU; menadione dimethylpyrimidinol bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13.2 mg; niacin, 39.6 mg; choline chloride, 500 mg; vitamin B₁₂, .022 mg; ethoxyquin, 125 mg; Mn, 55 mg; Fe, 80 mg; Cu, 4 mg; Zn, 40 mg; I, .35 mg; Se, .2 mg.

^dDry matter basis, crude protein and metabolizable energy calculated, dry matter and minerals determined by analysis.

fed a high Zn diet. Twenty-seven, day-old, feather-sexed (Ross x Ross) female chicks were reared in a heated Petersime battery and assigned randomly to units with raised floors made of galvanized steel.

Chicks were maintained on a 24 h constant-light cycle and allowed ad libitum access to feed and tap water. A completely randomized design was used with one replicate of nine birds assigned to three treatments.

Dietary treatments included the unsupplemented basal (zero added Zn) and the basal supplemented with 1000 mg/kg Zn as either RG acetate or RG oxide. The basal diet contained 63 mg/kg Zn (dry basis) by analysis and was formulated to meet the requirements of the starting chick (NRC, 1984).

Chicks were killed by cervical dislocation at 1, 2 and 3 wk of age. Bone was collected and frozen for subsequent Zn analysis as indicated for Experiment 8. Liver and pancreas were excised and frozen (-20°C) until further analysis of MT was performed as indicated for Experiment 1.

Statistical analysis of the data were performed by one-way ANOVA using the GLM procedure of SAS (1985). Treatment means were compared using single degree of freedom contrasts (Steel and Torrie, 1980).

Results

Experiment 8

The results of chick performance are summarized in Table 8-2. At 1 wk of age, daily feed intake ($P<.0529$), daily gain ($P<.0125$) and feed conversion ($P<.0419$) were influenced by dietary Zn concentration. At 2 wk of age, daily intake was drastically decreased ($P<.0135$), and daily gain was affected ($P<.0057$) as well as feed conversion influenced ($P<.0422$) by dietary Zn concentration. The lowest daily intake was observed for gluconate. When chick performance was evaluated at 3 wk of age daily intake ($P<.0061$) and daily gain ($P<.0027$) were decreased by dietary Zn intake. However, feed conversion was not affected, only numerical differences were observed.

Bone mineral concentration is presented in Table 8-3. Bone Zn was increased ($P<.0011$) by dietary Zn concentration. The largest bone Zn increase was observed for gluconate, 1.47 fold, compared to the basal treatment. On the other hand, sulfate showed a bone Zn increase of 1.35 fold compared to the basal treatment. Bone Cu and Fe concentration were not affected by dietary Zn concentration.

To estimate the relative biological availability value for gluconate, the bone Zn of sulfate (474 mg/kg), the standard source, was set as 100% bioavailable. Using mean ratio of bone Zn concentration from gluconate (516 mg/kg) to

TABLE 8-2. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON FEED INTAKE, GAIN AND FEED CONVERSION IN CHICKS, EXP. 8

Zinc source ^a	Added Zn, mg/kg	Daily intake, g	Daily gain, g	Feed/gain
Age, 1 wk ^b				
Basal	0	15.5	13.2	1.17
Sulfate	1000	14.8	8.3	1.78
Gluconate	1000	13.2	6.5	2.03
SE ^c		.22	.4	.08
ANOVA,		P value		
Source		.0529	.0125	.0419
Contrasts				
Basal vs Gluconate		.0248	.0059	.0211
Sulfate vs Gluconate		.0651	.1636	.2492
Age, 2 wk ^b				
Basal	0	40.7	27.2	1.50
Sulfate	1000	31.5	15.5	2.04
Gluconate	1000	25.6	9.0	2.87
SE ^c		.9	.8	.1
ANOVA,		P value		
Source		.0135	.0057	.0247
Contrasts				
Basal vs Others		.0073	.0029	.0198
Sulfate vs Gluconate		.0719	.0434	.0422
Age, 3 wk ^b				
Basal	0	56.9	39.1	1.46
Sulfate	1000	41.5	26.1	1.59
Gluconate	1000	32.1	19.5	1.64
SE ^c		1.1	.7	.05
ANOVA,		P value		
Source		.0061	.0027	.3530
Contrasts				
Basal vs Others		.0033	.0013	.1973
Sulfate vs Gluconate		.0391	.0259	.6330

^aBasal diet contained 62 mg/kg Zn dry basis by analysis.^bEach mean value represents 2 pens of eight chicks, except basal one pen of eight chicks.^cPooled standard error.

TABLE 8-3. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN TISSUE OF CHICKS, EXP. 8

Zinc source ^a	Added Zn, mg/kg	Mineral		
		Zn	Cu	Fe
Bone, mg/kg ^b (ash basis)				
Basal	0	350	8.3	288
Sulfate	1000	474	7.1	301
Gluconate	1000	516	4.2	285
SE ^c		14.9	.7	9.8
ANOVA, Source	P value			
Contrasts		.0011	.0774	.7468
Basal vs Sulfate		.0046	.0434	-
Basal vs Gluconate		.0003	.5467	-
Sulfate vs Gluconate		.2169	.0774	-
Liver, mg/kg ^b (dry basis)				
Basal	0	85	22.0	407
Sulfate	1000	94	20.3	339
Gluconate	1000	85	21.0	419
SE ^c		2.8	.4	21.7
ANOVA, Source	P value			
		.3095	.2127	.2307
Kidney, mg/kg ^b (dry basis)				
Basal	0	80	20.6	233
Sulfate	1000	81	34.7	271
Gluconate	1000	80	38.5	275
SE ^c		3.0	1.4	13.8
ANOVA, Source	P value			
Contrasts		.9330	.0002	.5090
Basal vs Others		-	.0001	-
Sulfate vs Gluconate		-	.2400	-

TABLE 8-3-- Continued

Zinc source ^a	Added Zn, mg/kg	Mineral		
		Zn	Cu	Fe
Pancreas, mg/kg ^b (dry basis)				
Basal	0	102	46.9	125
Sulfate	1000	252	24.3	123
Gluconate	1000	177	33.1	148
SE ^c		11.6	2.2	5.9
ANOVA,	P value			
Source		.0001	.0016	.2000
Contrasts				
Basal vs Others		.0002	.0008	-
Sulfate vs Gluconate		.0156	.1178	-
Brain, mg/kg ^d (dry basis)				
Basal	0	59	22.1	104
Sulfate	1000	56	29.8	139
Gluconate	1000	58	27.9	144
SE ^c		1.6	1.6	8.3
ANOVA,	P value			
Source		.7736	.2110	.1979

^aBasal diet contained 62 mg/kg Zn dry basis by analysis.

^bEach mean value represents eight chicks for the basal treatment and 16 chicks for the Zn sources.

^cPooled standard error.

^dEach mean value represents three pool samples for each treatment.

sulfate gave a bioavailability value of 108.8% for gluconate.

Tissue Zn, Cu and Fe uptake are shown in Table 8-3. As observed in previous experiments liver Zn, Cu and Fe uptake were not influenced by dietary Zn concentration.

Uptake of Zn and Fe by kidney were not affected by dietary Zn intake; however, kidney Cu concentration was ($P < .0002$) increased by dietary Zn concentration. As the level of Zn increased more Cu accumulated in kidney.

Pancreas Zn was increased ($P < .0001$) by dietary Zn concentration. A larger increase was observed for sulfate than gluconate (252 vs 177 mg/kg). Copper concentration in pancreas decreased ($P < .0016$) as more Zn was ingested. Pancreas Fe uptake was not influenced by dietary Zn concentration. Zinc, Cu and Fe accumulation in brain were not affected by dietary Zn intake.

Experiment 9

Bone mineral concentration as influenced by source of Zn and age of the bird are presented in Table 9-1. Bone Zn uptake was influenced by source of Zn ($P < .0001$) and age ($P < .0015$). Contrast of bone Zn from the basal treatment vs bone Zn from the two Zn sources showed ($P < .0001$) effect of Zn source. However, when both Zn sources were compared (acetate vs oxide) the difference was not significant. The largest bone Zn increases were observed during first 2 wk

TABLE 9-1. EFFECTS OF SOURCE AND LEVEL OF DIETARY ZINC ON MINERAL CONCENTRATION IN BONE OF CHICKS, EXP. 9

Zinc source ^a	Added Zn, mg/kg	Age wk ^b	Bone, mg/kg ^c		
			Zn	Cu	Fe
Basal	0	1	376	4.7	421
		2	371	5.8	280
		3	353	7.0	303
Acetate	1000	1	575	1.4	401
		2	614	4.1	428
		3	456	4.1	315
Oxide	1000	1	603	3.3	522
		2	502	4.5	347
		3	444	3.5	283
SE ^d			9.8	.6	14.6
ANOVA,		P value			
Source (S)			.0001	.2133	.3219
Age (A)			.0015	.4456	.0023
S x A			.0507	.9400	.1378
Contrast of sources					
Basal vs Others			.0001	.0886	.1380
Acetate vs Oxide			.1948	.7210	.9394
Contrast of age (wk)					
wk 1 vs wk 2 & 3			.0088	.2107	.0010
wk 2 vs wk 3			.0046	.9353	.1721
wk 1 & 2 vs wk 3			.0005	.4806	.0050

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.^bAge of birds in weeks at which they were killed.^cAsh weight basis, each value represents three chicks.^dPooled standard error.

of age of the birds compared to 3 wk of age ($P<.005$).

Bone Cu concentration was not affected by source or age of the bird. However, bone Fe was influenced ($P<.0023$) by the age of the bird. More Fe accumulated in bone during the first wk of age than the following 2 wk of age. However, as the bird got older and consumed more dietary Zn bone Fe concentration decreased during 2 and 3 wk of age.

Liver and pancreas MT concentrations as affected by dietary Zn concentration and age of the bird are presented in Table 9-2. Accumulation of MT in liver ($P<.0048$) and pancreas ($P<.0069$) were affected by a source x age interaction. Liver MT concentration at all ages were greater than pancreas MT concentration. As the bird got older more MT was generally accumulated in both tissues as a response to dietary Zn intake. Ingestion of Zn acetate caused more MT accumulation in liver and pancreas than Zn oxide. However, it was observed that MT accumulation in both tissues was not significant from 2 to 3 wk of age.

Discussion

The results of chick performance indicated that feeding high Zn diets for 3 wk caused more harmful effects in terms of decreased feed intake and reduced growth. It has been stated that highly bioavailable Zn sources are more toxic when ingested at high levels. This appeared to be the case for gluconate which decreased feed intake to a greater

TABLE 9-2. EFFECTS OF SOURCE AND DIETARY ZINC ON LIVER AND PANCREAS METALLOTHIONEIN ACCUMULATION IN CHICKS, EXP. 9

Zinc source ^a	Added Zn, mg/kg	Age wk ^b	Liver MT $\mu\text{g/g}^c$	Pancreas MT $\mu\text{g/g}^c$
Basal	0	1	28.1	30.7
		2	35.9	26.1
		3	31.4	30.0
Acetate	1000	1	50.2	41.3
		2	116.9	77.7
		3	139.6	78.2
Oxide	1000	1	48.5	47.7
		2	96.1	54.1
		3	98.0	50.9
SE ^d			3.2	1.8
ANOVA,		P value		
Source (S)			.0001	.0001
Age (A)			.0001	.0129
S x A			.0048	.0069
Contrast of sources				
Basal vs Others			.0001	.0001
Acetate vs Oxide			.0133	.0038
Contrast of age (wk)				
wk 1 vs wk 2			.0001	.0105
wk 1 vs wk 3			.0001	.0088
wk 2 vs wk 3			.4045	.9334

^aBasal diet contained 63 mg/kg Zn dry basis by analysis.

^bAge of the birds in weeks at which they were killed.

^cEach value represents three chicks, MT concentration is expressed as $\mu\text{g/g}$ fresh tissue.

^dPooled standard error.

extent than sulfate. Magee and Spahr (1964) indicated that a high level of protein may accentuate, rather than reduce, the severity of zinc toxicity in rats under certain conditions. It is possible that a situation similar to this may have occurred with those birds dosed orally and allowed to eat the basal diet that contained 23% protein.

On the other hand, some investigators reported that the depressed growth caused by the reduction in feed intake associated with Zn-deficiency caused a decrease in diadenosine tetraphosphate concentrations in liver and spleen of rats (Chesters et al., 1990) but this has not yet been investigated with chicks. Therefore, the biochemical mechanism of how high dietary Zn decreases feed intake and subsequent growth still remains unclear.

If bioavailability studies are to be conducted using practical diets supplemented with high levels of Zn it would be recommended to utilize 2-wk old chicks that have been previously fed practical diets. It appears that the detrimental effect of high levels of Zn is less pronounced during the third wk of life of the bird. Feeding high Zn diets for a short period of time, from 2 to 3 wk of age, would be sufficient to evaluate tissue Zn uptake especially bone Zn uptake, and use this parameter for determining Zn bioavailability.

Results from tissue Zn uptake were similar to those observed in previous experiments. Bone and pancreas were

the two tissues that accumulated more Zn compared to liver and kidney. It has been reported that Zn from bone can be utilized when Zn deficiency occurs (Harland et al., 1975); thus bone Zn constitute a functional reserve that is mobilized as it was shown in Experiment 6 and also reported by Brown et al. (1978) and Calhoun et al., (1978) who reported at least some mobilization of bone Zn from growing quail and rats fed excess Zn.

The Zn homeostatic mechanism functioned in the young chick as demonstrated by the no variation of Zn content in liver and kidney. However, pancreas seems to be very sensitive to excess Zn and similar results were reported for chicks by Lu and Combs (1988). The same investigators indicated that chicks fed practical diets did not achieve pancreas Zn levels of the magnitude associated with pancreatic exocrine dysfunction even when supplements containing or great as 2000 mg/kg Zn were fed for 10 d. Therefore, it is apparent that the toxicity of dietary Zn can be significantly influenced by factors that also can affect its nutritional bioavailability. The practical corn-soybean meal diet which contains such substances as phytate that renders Zn bioavailability low, offers substantial protection from levels of the mineral that can otherwise be toxic.

There is relatively little information available about the transport of Zn between the systemic circulation and the

brain (Kasarkis, 1984). The diffusion constant and linear binding constant are lower than for most other tissues (Jain et al., 1981). In the chick, the Zn content of brain was reported to be 12 $\mu\text{g/g}$ fresh tissue (Zeigler et al., 1964). In humans, at least three Zn-binding proteins have been identified in brain. These have apparent molecular weights of 15000, 25000, 210000 Da (Itoh et al., 1983). The lowest molecular weight protein could be MT as it is inducible under appropriate conditions (Ebadi et al., 1984). In the case of the chick these proteins have not been clearly identified and how they may relate to Zn transport across the blood-brain barrier and influence uptake by brain cells is not known.

With regard to MT accumulation in liver and pancreas, our data indicated that there was a trend to accumulate more Zn-MT in liver than pancreas especially for the first 2 wk of age. Fleet et al. (1988) reported higher pancreas Zn-MT than liver Zn-MT in chicks 24 h after an oral dose of Zn. The results from our study are based on a 3 wk period in which chicks were fed practical diets supplemented with 1000 mg/kg Zn. It is also possible that prolonged storage of samples may have caused degradation of intracellular MT. No current information is available about the decay of this protein under frozen conditions. Furthermore, Zn-MT has been reported to be susceptible to lysosomal breakdown (Feldman et al., 1978) , however, clear information about

this process in different tissues of chicks has not been reported. Oh et al. (1979) working with chicks fed purified diets for 5 wk and supplemented with high levels of Zn reported that Zn-MT gradually accumulated in tissues when chicks were fed up to 2000 mg/kg Zn in the diet but these investigators did not measure Zn-MT accumulation weekly as in the present experiment. The addition of 4000 mg/kg Zn to the diet markedly increased the Zn content of MT (Oh et al., 1979).

The significant difference in Zn-MT accumulation in liver and pancreas may be related to bioavailability of source. Zinc acetate is more bioavailable than Zn oxide. Thus more Zn from the acetate is transported from the portal blood to the liver which in turn triggers the synthesis of MT. The greater amount of Cu in kidney of chicks fed sulfate and gluconate may be related to the relative affinity of MT for Cu (Hamer, 1986).

In summary the findings of these experiments are three fold: first, feeding high Zn diets for 3 wk reduced chick performance especially if the Zn source was highly bioavailable; second, bioavailability studies using high Zn diets should be conducted with 2-wk old chicks; and third, the liver has a greater ability to accumulate Zn-MT than the pancreas on a per gram of tissue fresh basis when chicks are fed practical diets supplemented with high levels of Zn.

Summary

Two experiments were conducted with the following objectives 1) to investigate tissue Zn, Cu and Fe uptake as influenced by dietary Zn concentration, and 2) to examine Zn-MT accumulation in liver and pancreas of chicks fed practical corn-soybean meal diets supplemented with a high level of Zn for 2 wk. In Experiment 8, 40, day-old feather sexed (Ross x Ross) female chicks were assigned randomly to pens in a thermostatically controlled electrically heated Petersime battery. Chicks were maintained on a 24 h constant-light cycle and allowed ad libitum access to feed and tap water. A completely randomized design was used, with one replicate of eight birds for the basal treatment and two replicate pens of eight birds each for the two Zn sources. Treatments included the unsupplemented basal (62 mg/kg Zn) and the basal supplemented with 1000 mg/kg Zn as either RG sulfate or FG gluconate. Body weight of individual chicks and feed intake by pen were measured weekly. At 1-wk of age, daily feed intake ($P < .0529$), daily gain ($P < .0125$) and feed conversion ($P < .0419$) were influenced by dietary Zn concentration. At 2-wk of age, daily intake was drastically decreased ($P < .0135$), daily gain reduced ($P < .0434$) and feed conversion was affected ($P < .0422$) by elevated dietary Zn concentration. Similar patterns were observed for chick performance at 3-wk of age, daily intake ($P < .0061$) and daily gain ($P < .0027$) were decreased by dietary

Zn concentration but feed conversion was not affected. Results of tissue mineral concentration indicated that bone Zn uptake was increased ($P<.0001$) by dietary Zn concentration and by age of the bird ($P<.0015$). The largest bone Zn uptake was observed in chicks fed gluconate, 1.47 fold increase followed by sulfate 1.35 fold increase compared to the basal treatment. More bone Zn accumulated in bone during the first 2 wk of age compared to the third week of age. Bone Cu and Fe concentrations were not affected by dietary Zn intake. Using the mean-ratio of bone Zn (gluconate:sulfate) with sulfate set at 100%, the bioavailability value for gluconate was 108.8%. Liver Zn, Cu and Fe uptakes were not influenced by dietary Zn concentration. Kidney Zn and Fe uptakes were not influenced by Zn source; however, kidney Cu increased ($P<.0002$) as the level of Zn increased in the diet. Pancreas Zn was increased ($P<.0001$) by dietary Zn intake. Pancreas Cu concentration decreased ($P<.0016$) as more Zn was ingested. Pancreas Fe was not influenced by dietary Zn intake. Brain Zn, Cu and Fe concentrations were not affected by dietary Zn concentration. In Experiment 9, 27, day-old, feather-sexed (Ross x Ross) female chicks were allotted randomly to three treatments which included the unsupplemented basal (63 mg/kg Zn) and the basal supplemented with 1000 mg/kg Zn as either RG acetate or RG oxide. Husbandry of the chicks was similar to Experiment 8. Bone Zn uptake was influenced ($P<.0001$) by

source and age of the bird ($P < .0015$). Bone Cu was not affected by source or age of the bird. Bone Fe was decreased ($P < .0023$) by age of the bird. The decrease in bone Fe concentration was markedly observed after the first wk of age. Results of liver and pancreas MT accumulation indicated an increase ($P < .01$) by the source x age interaction. Liver MT concentration at all ages was higher than pancreas MT concentration. Ingestion of Zn acetate caused more MT accumulation in liver and pancreas than Zn oxide.

CHAPTER 8 GENERAL SUMMARY AND CONCLUSIONS

Nine experiments were conducted to investigate the relative biological availability (BV) of Zn from reagent grade (RG) and feed grade (FG) sources for sheep and poultry when added at high or low levels to practical diets. In Experiment 1, 42 crossbred wethers averaging 40 kg initially were used to determine the BV of Zn from RG sources. Animals were assigned randomly to seven treatment groups in a completely randomized design. The basal diet was a corn-soybean meal-cotton seed hulls diet (44 mg/kg Zn, dry basis by analysis). The diets were supplemented with 0, 700, 1400 or 2100 mg/kg Zn from $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (sulfate) or 1400 mg/kg Zn from $3\text{Zn}(\text{OH})_2 \cdot 2\text{ZnCO}_3$ (carbonate), ZnO (oxide) and Zn metal (metal), added at the expense of cornstarch. Lambs were fed the treatment diets for 23 d following a 7-d adjustment period during which all animals were fed the basal diet. Feed intake was not affected by high dietary Zn levels, and final body weight was not influenced by treatments. Liver Zn concentration was increased ($P < .001$) by high dietary Zn intake. However, liver Cu and Fe concentrations were not affected by dietary Zn concentration. Zinc and Cu uptake by kidney were increased ($P < .001$) by dietary Zn intake, but Fe

concentration was not affected. Pancreas Zn and Cu uptake were increased ($P<.001$) by dietary Zn intake; however, pancreas Fe was not affected. Multiple linear regression (MLR) of log transformed tissue Zn on dietary Zn concentration indicated that the increase was linear for liver ($P<.0001$; $R^2=.77$), kidney ($P<.0001$; $R^2=.92$), and for pancreas ($P<.0001$; $R^2=.87$). By means of MLR and slope ratio methodology BV were estimated for the RG sources with sulfate, set at 100%. A BV index, calculated using the mean of the estimates from the different tissues, provided values of 106.4, 105.8 and 76% for the carbonate, oxide and metal sources, respectively.

In Experiment 2, 42 crossbred wether lambs averaging 37.5 kg were used to determine the BV of Zn from RG sources. Animals were allotted randomly to seven treatment groups in a completely randomized design. The basal diet was a corn-soybean meal-cotton seed hulls diet containing 64 mg/kg Zn (dry basis) by analysis. The diets were supplemented with 0, 700, 1400 or 2100 mg/kg Zn from sulfate-A, or 1400 mg/kg Zn from sulfate-B, oxide-A and oxide-B, added at the expense of cornstach. Feed consumption and body weight were not affected by treatments. Liver Zn and metallothionein (MT) concentrations increased ($P<.001$) as dietary Zn intake increased. Liver Zn and liver MT concentration were highly correlated $r=.96$. Liver Cu tended to increase ($P<.056$) as dietary Zn increased, but liver Fe decreased ($P<.012$) as the

dietary Zn intake increased. Kidney Zn and Cu uptake increased ($P<.01$) as dietary Zn increased, but kidney Fe was not affected by the treatments. Pancreas Zn was influenced ($P<.001$) but Cu and Fe were not affected by dietary Zn intake. MLR of log transformed tissue Zn on dietary Zn by source indicated that the increase was linear for liver ($P<.0001$; $R^2=.78$); kidney ($P<.0001$; $R^2=.81$) and for pancreas ($P<.0001$; $R^2=.78$). By means of MLR and slope ratio methodology, sulfate-A was set at 100%, BV were estimated for the FG sources. A BV index calculated using the mean of the estimates from the different tissues, gave values of 85.7, 87.2 and 79.2% for sulfate-B, oxide-A and oxide-B sources, respectively. In conclusion, data indicated that tissue uptake of Zn in lambs fed high dietary Zn levels for a short period of time in practical diets offers a practical methodology to assess the BV of Zn from RG and FG Zn sources for sheep. RG sulfate, carbonate and oxide provided more bioavailable Zn than metal (100% vs 76%). However, FG sulfate-B, oxide-A and oxide-B had almost similar BV values (79-87%).

Experiment 3 was conducted to investigate the BV of Zn from RG sources for chicks; 273 day-old feather-sexed chicks were allotted randomly to 13 treatments which include an unsupplemented basal corn-soybean meal diet (63 mg/kg Zn) or the basal supplemented with 400, 800 or 1200 mg/kg Zn from RG sulfate (22.0% Zn), carbonate (58.5% Zn), oxide (77.4%

Zn) or metal (95.5% Zn). Chicks fed sulfate showed the poorest performance, consumed less feed and had lower gains than those fed carbonate, oxide, metal or basal diets. Liver Zn was increased ($P<.0001$) by dietary Zn concentration. Liver Cu was not decreased by dietary Zn or source; however, liver Fe was influenced ($P<.0182$) by the source x level interaction. An interaction ($P<.0369$) was observed for kidney Zn; kidney Cu was not influenced by dietary Zn or source but kidney Fe was affected ($P<.012$) by dietary Zn concentration. MLR analysis of bone Zn concentration with respect to dietary Zn concentration indicated a significant ($P<.0001$; $R^2=.62$) linear relationship between these two variables. Using the slope ratio technique with sulfate set at 100%, the BV were 77.7, 76.8 and 45.8% for carbonate, oxide and metal sources, respectively. When bone Zn concentration was regressed on dietary Zn intake, the relationship between these two variables was also significant ($P<.0001$; $R^2=.48$). The BV were 57.1, 54.0 and 29.8% for carbonate, oxide and metal sources respectively. The data indicated that BV of Zn from RG sulfate was higher than RG carbonate and oxide; metal was the least bioavailable.

In Experiment 4, 288 day-old feather-sexed chicks were used to estimate the BV of Zn from FG sources. Chicks were allotted randomly to 16 treatments, which included an unsupplemented basal corn-soybean meal diet (75 mg/kg Zn) or

the basal supplemented with 300, 600 or 900 mg/kg Zn as either RG sulfate (22.0% Zn), FG sulfate-A (33.0% Zn), FG sulfate-B (32.2% Zn), FG oxide-A (72.0% Zn) or FG oxide-B (71.0% Zn). Feed intake and gain were influenced by a source x level interaction ($P < .0066$). Birds fed RG sulfate, FG sulfate-A and FG sulfate-B showed the lowest performance compared to other treatments. Bone Zn ($P < .0074$) and Cu ($P < .0065$) were increased by a source x level interaction. Bone Fe was not influenced by dietary Zn or source. Liver Zn and Cu were not influenced by dietary Zn or source. A source x level interaction ($P < .0156$) was observed for liver Fe. Kidney Zn, Cu and Fe were not influenced by dietary Zn or source. Pancreas Zn uptake increased ($P < .004$) as dietary Zn increased. A source x level interaction ($P < .001$) was observed for pancreas Cu and Fe. MLR analysis of bone Zn concentration on dietary Zn concentration indicated a relationship ($P < .001$; $R^2 = .57$) between these two variables. Using slope ratio methodology with RG sulfate set at 100%, the BV were 99.3, 80.9, 77.6 and 53.9% for sulfate-A, sulfate-B, oxide-A and oxide-B sources, respectively. When bone Zn concentration was regressed on dietary Zn intake the BV were 77.6, 74.5, 52.2 and 37.7% for sulfate-A, sulfate-B, oxide-A and oxide-B sources, respectively. The data indicated that BV for the FG sources were sulfate-A > sulfate-B > oxide-A > oxide-B.

Experiment 5 was conducted to investigate the BV for chicks of Zn from FG sources fed at low dietary levels. 240, day-old feather-sexed chicks were assigned randomly to 10 treatments which include an unsupplemented basal corn-soybean meal diet (35 mg/kg Zn) or the basal supplemented with 40, 80 or 120 mg/kg Zn from sulfate-RG (22.0% Zn), FG sulfate-A (33.0% Zn) or FG oxide-A (72.0% Zn). Feed intake and gain were affected ($P < .012$) by source. Chicks fed the gradient levels of sulfate-RG consumed less feed and had lower gains compared to other treatments. However, feed conversion was not influenced by source or dietary Zn concentration. Bone Zn increased due to the effect of source ($P < .0267$) and dietary Zn concentration ($P < .0121$), but no further bone Zn increase was observed above 80 mg/kg Zn from all sources under study. Bone Cu was not influenced by source or dietary Zn. A source x level interaction ($P < .0001$) was observed for bone Fe uptake. Liver Zn, Cu and Fe uptakes were not affected by source or dietary Zn. Kidney Zn was increased ($P < .0233$) by Zn source. Kidney Cu and Fe concentrations were not affected by source or dietary Zn. Pancreas Zn uptake increased ($P < .0008$) as dietary Zn increased. Pancreas Cu and Fe concentrations were not affected by source or dietary Zn. The MLR analysis of bone Zn concentration on dietary Zn concentration indicated that this relationship was linear ($P < .0001$; $R^2 = .70$). Using slope ratio methodology with sulfate-RG set at 100%, the BV were

93.8 and 74.0% for the sulfate-A and oxide-A sources, respectively. When bone Zn concentration was regressed on dietary Zn intake the relationship between these two variables was linear ($P < .0001$; $R^2 = .68$). The estimated BV were 93.4 and 67.8% for the sulfate-A and oxide-A sources, respectively. Data indicated that BV of Zn from sulfate-A was higher than oxide-A (93% vs 67%).

Experiments 6 and 7, were conducted with chicks and the objectives were two fold 1) to examine the effect of a high dietary level of soluble sources of Zn on tissue Zn, Cu and Fe uptake as influenced by two routes of Zn administration, and 2) to estimate the BV of Zn using bone Zn uptake as the response criterion in chicks fed high Zn diets from 2 to 3 wk of age. In Experiment 6, 48, day-old male chicks were housed in Petersime batteries and were fed a practical corn-soybean meal diet for the first 2 wk of age. At 10 d of age chicks were assigned randomly to individual pens (1 bird per pen) to allow them to acclimate to their new surroundings. Individual feed intake was recorded daily for all birds during the acclimation period (10 to 14 d). At 14 d of age, one-half of all birds (24 birds) were assigned randomly to four dietary treatments (1, 2, 3 and 4) which included the basal corn-soybean meal diet (62 mg/kg Zn) or the basal supplemented with 1000 mg/kg Zn from RG sulfate (22.0% Zn), acetate (29.2% Zn) or chloride (45.8% Zn). The other 24 birds were assigned randomly to four treatments (5, 6, 7 and

8) which included the basal corn-soybean meal diet plus a single oral dose (crop intubated) of water or the basal supplemented with an oral dose of 1000 mg/kg Zn from RG sulfate, acetate or chloride. Feed intake was recorded daily for each chick from d 14 to d 21 of the study. Chicks that received an oral dose of Zn decreased ($P<.0001$) feed intake at 24 h post-oral dose compared to chicks fed either the control diet or fed high Zn diets. After the oral dose of Zn was stopped, feed intake was similar for all treatments. Bone Zn evaluated at 168 h post-treatment was increased ($P<.0001$) by source, but bone Cu and Fe uptake were not affected by route of Zn administration or source. On the other hand, bone mineral uptake in chicks given an oral dose of Zn showed a source x post-treatment time interaction ($P<.0295$) for bone Zn concentration. This increase was larger at 24 h post-oral dose than at 168 h post-oral dose. Bone Cu and Fe uptakes were not affected by source or post-treatment time. Liver Zn uptake at 168 h post-treatment was not affected in chicks fed high Zn diets or in those given an oral dose of Zn; liver Cu uptake at 168 h post-treatment was influenced ($P<.0046$) by route of Zn administration but liver Fe was not affected by the route of Zn administration or source. In chicks given an oral dose of Zn, liver Zn measured at 24 h post-oral dose increased ($P<.0001$) by the effect of source x post-treatment time interaction, liver Cu increased ($P<.0201$) at 24 h post-

treatment but liver Fe was not affected by source or post-treatment time. Kidney Zn uptake at 168 h post-treatment indicated that kidney Zn was influenced ($P < .0110$) by a route of Zn administration x source interaction; kidney Cu was not affected by route of Zn administration or source, and kidney Fe concentration was increased ($P < .0220$) by route of Zn administration. Chicks given an oral dose of Zn showed an increase ($P < .001$) in kidney Zn due to the source x post-treatment time interaction; kidney Cu was increased ($P < .0001$) by post-treatment time, being higher at 24 h than 168 h, and kidney Fe was increased ($P < .0012$) by a source x post treatment time interaction. Pancreas mineral uptake was evaluated only in chicks that received an oral dose of Zn; pancreas Zn ($P < .0001$) and Fe ($P < .0026$) concentrations were increased by a source x post-treatment time interaction but pancreas Cu was not influenced by source or post-treatment time. Duodenal mucosal cells (IM) of chicks given an oral dose of Zn showed an increase ($P < .0026$) in Zn uptake by the IM due to the source x post-treatment time interaction. Uptake of Cu by the IM was increased ($P < .034$) by post treatment time. Iron uptake by the IM was not influenced by source or post-treatment time. Zinc concentration in the three segments of the small intestine was increased ($P < .0002$) by a source x post-treatment time interaction. Zinc uptake was higher in duodenum > jejunum > ileum. Copper uptake was influenced ($P < .0065$) by segment of

the small intestine being higher in duodenum and jejunum than ileum. Iron uptake by the small intestine was decreased by source ($P<.0407$), segment of the small intestine ($P<.0001$) and post treatment time ($P<.0029$). Ingestion of high levels of Zn decreased Fe uptake especially at the level of the duodenum and jejunum, respectively.

In Experiment 7, 48 day-old male chicks were fed a basal corn-soybean meal diet (63 mg/kg Zn). Husbandry of the birds during the first 2 wk was similar to Experiment 6. At 2 wk of age birds were assigned randomly to nine treatments in two groups. One group of birds included treatments 1, 2, 3 and 4; chicks were fed the basal diet or the basal supplemented with 1000 mg/kg Zn from RG sulfate, acetate or chloride. The other group of birds included treatments 5, 6, 7, 8 and 9; chicks were fed the basal diet plus oral dose (crop intubated) of water or 1000 mg/kg Zn from RG sulfate, acetate, chloride or FG gluconate. The oral doses of water or Zn were administered daily at 1000 h from 2 to 3 wk of age. Feed intake ($P<.0005$), daily gain ($P<.0001$) and feed conversion ($P<.0016$) of chicks given oral doses of Zn were decreased compared to those chicks fed high Zn diets. Feed intake ($P<.0251$) and daily gain ($P<.0024$) were influenced by a route of Zn administration x source interaction. Feed conversion was decreased by the route of Zn administration and source. Bone Zn

concentration was increased ($P<.001$) by a route of Zn administration x source interaction. The highest bone Zn uptake was observed in those chicks given oral doses of Zn compared to those fed high Zn diets. Bone Cu and Fe were not affected by route of Zn administration or source. Mean ratio of bone Zn concentration gave BV of 105.7 and 101.2 % for RG acetate and chloride sources, respectively. When mean bone Zn concentration was used for chicks given oral doses of Zn BV were 117.9, 105.5 and 105.2% for RG acetate, chloride and FG gluconate sources, respectively. Liver Zn concentration was increased ($P<.0003$) by a route of Zn administration x source interaction. The greatest liver Zn uptake was observed in chicks given oral doses of Zn. Liver Cu was influenced ($P<.0185$) by the same interaction. Liver Fe concentration was decreased ($P<.0001$) by route of Zn administration. Chicks given oral doses of Zn had lower ($P<.0001$) liver Fe than those fed high Zn diets. Kidney Zn ($P<.0001$) and Fe ($P<.0004$) were influenced by a route of Zn administration x source interaction, similarly kidney Cu was influenced ($P<.0248$) by the same interaction. More Zn and Cu were accumulated in kidney of chicks given oral doses of Zn than those fed Zn diets. Kidney Fe was decreased in chicks dosed orally with Zn. Pancreas Zn was increased ($P<.0001$) by a route of Zn administration x source interaction. Chicks given oral doses of Zn had the highest pancreas Zn concentration. Pancreas Cu and Fe were not

affected by route of Zn administration or source. Brain Zn and Cu were not influenced by route of Zn administration or source. However, brain Fe decreased ($P<.0114$) in chicks given oral doses of Zn especially those chicks given RG acetate and chloride. Data from experiments 6 and 7 indicated that single daily oral doses of high levels of Zn cause more detrimental effects on performance of chicks compared to those fed high levels of Zn in the diet ad libitum. For future BV studies, use of 2-wk old chicks would be recommended with high Zn diets fed from 2 to 3 wk of age. Comparable BV values were obtained for the RG sources evaluated under the two different routes of Zn administration.

Experiment 8 was conducted to examine chick performance and tissue uptake of Zn, Cu and Fe when fed high soluble Zn sources for 3 wk. Forty day-old feather-sexed (Ross x Ross) chicks were allotted randomly to three treatments in a completely randomized design, with one replicate of eight birds for the basal treatment and two replicate pens of eight birds each for the two Zn sources. Treatments included the unsupplemented basal (62 mg/kg Zn) and the basal supplemented with 1000 mg/kg Zn as either RG sulfate or FG gluconate. At 1-wk of age, daily feed intake ($P<.0529$), daily gain ($P<.0125$) and feed conversion ($P<.0419$) were affected by dietary Zn concentration. At 2-wk of age, daily intake was drastically decreased ($P<.0135$),

daily gain reduced ($P<.0057$) and feed conversion was affected ($P<.0247$) by dietary Zn concentration. Similar patterns were observed for chick performance at 3-wk of age, daily intake ($P<.0061$) and daily gain ($P<.0027$) were decreased by dietary Zn concentration but feed conversion was not affected. Results of tissue mineral concentration indicated that bone Zn uptake at 3-wk of age was increased ($P<.0011$) by dietary Zn concentration. The largest bone Zn uptake was observed in chicks fed gluconate, 1.47 fold increase followed by sulfate 1.35 fold increase compared to the basal treatment. Bone Cu and Fe concentrations were not affected by dietary Zn intake. Using the mean ratio of bone Zn (gluconate:sulfate) with sulfate set at 100%, the BV for gluconate was 108.8%. Liver Zn, Cu and Fe uptakes were not influenced by dietary Zn concentration. Kidney Zn and Fe uptakes were not influenced by Zn source; however, kidney Cu increased ($P<.0002$) as the level of Zn increased in the diet. Pancreas Zn was increased ($P<.0001$) by dietary Zn intake. Pancreas Cu concentration decreased ($P<.0016$) as more Zn was ingested. Pancreas Fe was not influenced by dietary Zn intake. Brain Zn, Cu and Fe concentrations were not affected by dietary Zn concentration. Data indicated that high levels of Zn from highly soluble sources decreased feed intake and subsequent growth. The BV value for gluconate was numerically higher than that for sulfate.

Experiment 9 was conducted to examine bone Zn concentration and MT accumulation in liver and pancreas in chicks fed high levels of Zn for 3 wk. Chicks were allotted randomly to three treatments which included the unsupplemented basal (63 mg/kg Zn) and the basal supplemented with 1000 mg/kg Zn as either RG acetate or RG oxide. Bone Zn uptake was influenced ($P<.0001$) by source and age of the bird. Bone Cu was not affected by source or age of the bird. Bone Fe was decreased ($P<.0041$) by age of the bird. The decrease in bone Fe concentration was markedly observed after the first wk of age. Liver MT ($P<.0048$) and pancreas MT ($P<.0069$) accumulation increased by a source x age interaction. Liver MT concentrations at all ages were higher than pancreas MT concentrations. Ingestion of Zn acetate caused more MT accumulation in liver and pancreas than Zn oxide. Data indicated that bone Zn increased rapidly due to intake of high levels of Zn; thus for BV studies 1 wk feeding with high levels of Zn may provide bone Zn uptake to estimate the BV of Zn from Zn sources.

In conclusion, data from sheep experiments indicated that it is feasible to determine bioavailability of Zn from reagent grade and feed grade sources when they are fed to lambs at high but nontoxic levels in practical diets for a short period of time. No detrimental effects were observed in terms of feed consumption and body weight of the animals.

The bioavailability values of Zn estimated for the different sources could be considered adequate and may be applicable to better feed formulation, and use of supplemental zinc sources for sheep.

With regard to the data obtained from chick experiments, it can be concluded that high levels of Zn from highly soluble sources cause detrimental effects on performance of chicks in terms of reduction in feed consumption and subsequent growth. Not all sources were detrimental despite that they were supplemented to provide high dietary Zn concentration. However, for future bioavailability studies if this methodology is used it would be recommended to feed high levels of Zn to chicks from 2 to 3 wk of age. The accumulation of Zn in tissues such as bone, during this period, is sufficient for determining bioavailability of Zn from reagent or feed grade sources. The bioavailability values obtained using this methodology are comparable with those bioavailability values obtained when low levels of Zn were added to practical diets. The Zn bioavailability values obtained for the different feed grade sources provide essential information for the practical nutritionist to select the most appropriate source for feed formulation.

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BIOGRAPHICAL SKETCH

Manuel Sandoval Chacón was born in Tingo Maria, Peru, on March 21, 1954. He attended Gómez Arias Dávila High School in Tingo Maria and completed his high school education in 1970. The following year he entered the Universidad Nacional Agraria de la Selva, Tingo Maria and was awarded the degree of Bachelor of Science and Zootechnical Engineering in June, 1976. Following graduation he worked as an extension agent for the Minister of Agriculture until March 1977. From April 1977 to 1979 he worked as assistant professor in the Department of Animal Science at the Universidad Nacional Agraria de la Selva.

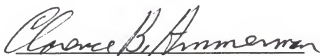
In January, 1980 the author was awarded a scholarship from LASPAU/AID and began graduate studies in the area of poultry nutrition at the University of Missouri. He received the degree of Master of Science in poultry science in December, 1982. Then he returned to the Universidad Nacional Agraria de la Selva, where he was involved in teaching and research until 1988.

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He is married to the former Evi Luz Aliaga and has two daughters, Evelyn C. and Mary S.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Clarence B. Ammerman, Chairman
Professor of Animal Science

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Joseph H. Conrad
Professor of Animal Science

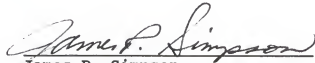
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May 1992


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